ADVANCES IN INTERNAL MEDICINE

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The Metabolism of Vitamin B_{12} in Pernicious and Other Megaloblastic Anemias*

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IT IS ONLY in the last 10 years that the indispensable role of extremely minute amounts of vitamin B_1 (cyanocobalamin) in normal metabolism has been unequivocally demonstrated Our knowledge of the nature of pernicious anemia and of other megaloblastic anemias has been increased greatly as a result of such studies The various concepts of pernicious anemia as (1) a toxic disease (2) a hemolytic disorder (3) a neoplastic process and (4) an infectious disease have now been discarded and its nature as a deficiency disease—first suggested on purely theoretic grounds by Flint (38) and first demonstrated by the therapeutic triumphs of Minot, Murphy and Castle (24 26 27 82) —is universally accepted

The levels of B_{12} in the body are measured in micromicrograms or at most in micrograms it is the inability of a person to absorb from the food even these minute amounts which causes pernicious anemia As Castle (25) has stated " this disease would not develop if the

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We wish to thank Merck and Company Rahway New York, and the Squibb Institute for Medical Research New Brunswick, New Jersey for their support of these studies and for generous supplies of test materials

blood plasma in the amount of 200 to 900 $\mu\mu$g per milliliter (41 67 84 106) From 5 to 30 per cent of the circulating B_1 is immediately available to the test organism and is therefore called free the remainder is assayed after heating the plasma to 100 C for 30 minutes a process which presumably separates it from the protein to which it is bound. This B_{12} is normally bound chiefly to the serum globulins Vitamin B_{12} is present in largest amounts in normal liver (80 112, 115 124) and in lesser amounts in kidney (80 112 115) muscle (111) and

TABLE 1 —SERUM LEVELS OF VITAMIN B_{12} IN VARIOUS DISORDERS

CONDITION	TOTAL B_{12}, $\mu\mu$G./ML.
Normal	200–900
Pernicious anemia	
Relapse	0–50
Remission parenteral therapy	200–900
Remission incomplete oral therapy	100–300
Total gastrectomy relapse	0–50
Malabsorption syndrome	200–900 or 50–100
Diphyllobothrium latum anemia	0–50
Blind loop anemia	0–50
Pernicious anemia of pregnancy	200–900
Posterolateral sclerosis with or without anemia	0–20
Megaloblastic anemia due to anticonvulsant drugs	—
Chronic myelocytic leukemia	1 500–17 000
Acute liver disease	800–6 000

other tissues (112) and in pleural ascitic joint and cerebrospinal fluids (84) Normally the urine contains some 0 2 μg per 24 hours all in the free form (84) The stool contains 3 to 20 μg both normally and in certain disease states including pernicious anemia (10 23 42) however some of the fecal B_1 is synthesized *de novo* by bacteria in the colon and is not available for absorption or tissue metabolism (10)

In pernicious anemia in relapse—the prototype of B_1 deficiency—the patient s body in contrast to that of the normal individual contains minimal amounts of B_1 The serum level approaches zero (67 84) the amount in the urine is greatly reduced (84) and the liver contains practically none (43 123) The serum B_{12} content may also be reduced in other megaloblastic anemias (sprue nutritional macrocytic anemia intestinal blind loops with anemia) but rarely to the same degree as in pernicious anemia On the other hand in certain megalo

patient could effect daily the transfer of a millionth of a gram of vitamin B_1 the distance of a small fraction of a millimeter across the intestinal mucosa and into the blood stream This he cannot do "

Vitamin B_{12} is a protoporphyrin like complex containing an atom of cobalt in its molecule (59 119) It plays a part in the basic synthesis of nucleoproteins and in the intermediate metabolism of carbohydrate and fat Its presence is essential to the normal development of cells throughout the body It is the purpose of the present discussion to examine the metabolism of this substance—its absorption transport utilization and excretion—in man and especially to clarify the mechanism by which B_1 deficiency occurs This deficiency leads to the development of megaloblastic anemia and other disorders

TECHNICS OF STUDY

Since the isolation of vitamin B_1 in 1948 and the acceptance of the fact that B_{12} is the extrinsic factor of Castle the laborious assay technics (81) using patients with pernicious anemia in relapse have been largely replaced by newer methods These new technics which measure B_{12} directly may be considered as falling into two categories *(1)* estimation of the amount of B_{12} in the blood and body tissues and *(2)* determination of the fate of B_1 by radioactive technics

Measurement of Vitamin B_{12} in Body (Table 1)

The order of magnitude of B_1 levels in body tissues is so low that chemical methods of determination are precluded and microbiologic assay technics are mandatory (12 34 39 51 61 62 84 88 101 109–111) Such technics are based on the use of bacteria and protozoa whose growth under specified conditions is a measure of the amount of B_{12} in the medium Older methods use Lactobacillus leichmannii and Escherichia coli (12 34 51 101 109) recently more accurate and more specific results have been obtained with the protozoan Euglena gracilis var bacillaris (62 67 84 110) and Ochromonas malhamensis (39 61) The microbiologic technics have given reliable data on the distribution of B_{12} in body tissues

By bioassay it has been determined that the B_{12} content of the average American diet approximates 5 µg daily so that an average meal contains 0 5 to 2 µg In the normal human body B_{12} circulates in the

their radioactivity The difference between the amount of radioactivity ingested and that excreted in the stool until no further radioactivity appears (approximately 7 days) is a measure of the amount of B_{12} absorbed The normal individual excreted 0 to 42 per cent of the oral dose The patients with pernicious anemia excreted virtually the entire oral dose (72-96 per cent) when the test was repeated later in patients with pernicious anemia who were given at the same time potent intrinsic factor the radioactivity in the stool fell toward normal values (14-29 per cent) Presumably the amount of (radioactive) B_{12} ab-

TABLE 2 —RADIOACTIVE TECHNICS FOR STUDY OF INTESTINAL ABSORPTION OF VITAMIN B_{12}

TEST	METHOD[a]	TISSUE OR EXCRETA EXAMINED	TIME	NORMAL[b]	PERNICIOUS ANEMIA[b]	
					Without I F	With I F
Heinle Welch	0 5 µg orally	Stool	For 7 d	0-42%	90-100%	0-42%
Glass	0.5 µg orally	Liver	After 5-7 d	+	–	+
Schilling	0 5 µg orally and 1 000 µg non radioactive intramuscularly	Urine	For 24 hr	8-40%	0-1%	8-40%
Blood	0.5 µg orally	Plasma	After 8-12 hr	±	–	+

[a] Radioactive vitamin B_{12} ($Co^{60}B_{12}$, $Co^{58}B_{12}$, $Co^{57}B_{12}$)
[b] + radioactivity – no radioactivity I F intrinsic factor

sorbed via the intestine rose to normal This distinction between the normal state and that of pernicious anemia—nonabsorption of a test oral dose of B_{12} in the absence of added intrinsic factor—held whether the patient was in clinical and hematologic relapse or remission

Subsequent studies (53 104) have confirmed the results obtained with the Heinle Welch technic

SCHILLING URINARY EXCRETION TECHNIC (66 116 117)—When a physiologic amount of radioactive B_{12} is given by mouth to a normal subject, virtually no radioactivity appears in the urine But if at about the same time a large dose (1 000 µg) of nonradioactive B_{12} is administered parenterally a detectable portion (8-40 per cent) of the radioactive (oral) dose appears in the 24 hour urine specimen (66 74 99 104 116 117) In patients with pernicious anemia under the same

blastic anemias (sprue pernicious anemia of pregnancy) the serum B_1 level may be normal (85 88 95)

Thus megaloblastic anemias may be associated with a normal or a reduced level of serum B_1 Since the serum level is probably a reflection of the tissue content of B_{12} (85) megaloblastic anemias may be of two types in this regard those with reduced stores of B_{12} and those with normal stores An obvious corollary borne out by clinical observations and experimental studies is that the development of megaloblastic erythropoiesis may be due to more than one factor in some cases to a tissue deficiency of B_1 in others to deficiencies of other substances notably folic acid and possibly ascorbic acid and other materials In general patients with megaloblastic anemias whose serum (and therefore tissue) levels of B_{12} are normal do not respond to B_{12} therapy while those with low serum levels do (85 87)

Radioactive Technics

By adding radioactive cobalt to the medium in which a culture of Streptomyces griseus is growing it is possible to induce the synthesis not only of normal (nonradioactive) B_1 but also of B_1 with a radioactive cobalt atom (25 118 120) In this way $Co^{56}B_{12}$ $Co^{58}B_1$ and $Co^{60}B_{12}$ have been produced and have been made available for tracer studies of the vitamin in the body All such studies have shown the same general result when B_1 is ingested in physiologic amounts its absorption depends on some gastrointestinal substance (intrinsic factor) present in the normal individual but lacking in the patient with pernicious anemia (25) When, however B_1 is ingested in larger than physiologic amounts a point is reached at which absorption occurs by a "mass action effect (31 113) not dependent on intrinsic factor This manner of absorption occurs not only in the normal person but also in the patient who lacks intrinsic factor i e in pernicious anemia Finally faulty intestinal absorption of B_1 may be present despite adequate production of intrinsic factor as part of a generalized mucosal (enzymic?) disorder which also includes impaired absorption of glucose vitamin A vitamin K, protein and other substances this is the condition in the malabsorption syndrome (76 99)

Several radioactive technics are of clinical importance (Table 2)

Stool method (Heinle Welch) (52) —The original technic for the study of B_1 absorption was to give the patient a physiologic (0 5 μg) dose of radioactive B_{12} by mouth collect all stools and determine

anemia and at least some patients with malabsorption syndrome how ever absorb virtually none

GLASS HEPATIC UPTAKE TECHNIC (49)—Both the Heinle-Welch and the Schilling technics measure B_1 absorption indirectly by deter mining the fraction of an oral dose which appears in the excreta Glass (49) has suggested a "direct" technic in which a physiologic amount of radioactive B_{12} is ingested and the body is then scanned *in vivo* to determine whether there is localization of radioactivity in the liver In *normal subjects* he found radioactivity to be present in the first few days throughout the abdominal cavity some of this came from the B_1 in the intestinal canal which ultimately passed into the feces After 6 or 7 days, however, all remaining radioactivity was localized in the right upper abdominal quadrant, indicating that the liver had taken up the ingested B_{12} Such hepatic uptake did not occur in the patients with pernicious anemia whether in remission or in relapse however when the test was repeated with the addition of intrinsic factor hepatic uptake—and therefore intestinal absorption—*of the ingested B_1 occurred* Again *certain patients with sprue* showed no hepatic uptake even with the use of intrinsic factor The hepatic radioactivity in the normal subjects and in the patients with pernicious anemia given intrinsic factor persisted for long periods of time possibly for years (117)

RADIOACTIVITY IN BLOOD—A fourth technic measures the presence of radioactivity in the blood after an oral tracer dose of B_1 (15 34) When normal subjects take radioactive B_1 of exceptionally high specific activity (1 000–5 000 μc/mg) by mouth radioactivity in the *blood can be demonstrated within hours it is present in the plasma* and becomes maximal in 8 to 12 hours In pernicious anemia radio activity cannot be detected in the blood but again if the test is re peated with added intrinsic factor radioactivity is found in the plas ma just as in the normal subject

All these methods lead to one general conclusion the absorption of physiologic amounts of vitamin B_{12} the amount present in an ordinary meal, for example normally depends on the presence of intrinsic fac tor in the gastrointestinal tract Absence of the factor causes the non absorption of ingested B_1 in pernicious anemia and in patients after total gastrectomy and absorption in both conditions can be effected by adding intrinsic factor to the oral B_1 However intestinal absorp tion of B_1 may be faulty for other reasons as discussed below

conditions no radioactivity is detectable in the urine when the test is repeated with the addition of intrinsic factor the urine shows radio activity the values approaching the normal range (99 116) In pa tients with malabsorption syndrome there may also be little or no urinary radioactivity but in such cases addition of intrinsic factor does not increase the radioactivity (99) However this defect does not appear in every patient with sprue nor do all types of megaloblastic

✓TABLE 3 —ABSORPTION OF ORAL DOSE OF RADIOACTIVE B_{12} IN MEGALOBLASTIC ANEMIAS

CONDITION	ABSORPTION WHEN GIVEN	
	Without Intrinsic Factor	With Int ns c F tor
Normal	Absorbed	No change
Pernicious anemia		
Relapse	Not absorbed	Absorbed
Remission	Not absorbed	Absorbed
Total gastrectomy	Not absorbed	Absorbed
Malabsorption syndrome	Absorbed not absorbed	No change not absorbed
Intestinal blind loop	Not absorbed[b]	Not absorbed
Diphyllobothrium latum anemia	Not absorbed	Not absorbed (?)
Pernicious anemia of pregnancy	Absorbed	?
Nutritional megaloblastic anemia	Absorbed	No change
Megaloblastic anemia of infancy	?	?
Megaloblastic anemia due to drugs	?	?

S footn t T bl 2
[b] After ant b ot c (t tracy lin blort t acy l n) therapy abs rpt n may be ome n rmal

anemia give abnormal results in the Schilling test, i.e. abnormal ab sorption of orally administered B_{12} (Table 3)

The appearance of radioactivity in the urine under the conditions of this test indicates that a portion of the orally administered radio active B_{12} has been absorbed has entered the blood and has subse quently been excreted into the urine Callender and Evans (22) have calculated that in the normal subject approximately 33 per cent of an absorbed oral dose of radioactive B_{12} appears in the urine under these conditions The normal subject therefore absorbs 27 to 100 per cent of the small test dose these figures correlate well with those of the Heinle Welch stool technic (56) The patient with pernicious

B_{12} ultimately produces tissue deficiency of B_{12} Such a patient can not absorb the physiologic amount of vitamin B_{12} present in food or in the radioactive test dose but when artificial concentrates of intrinsic factor are given together with the oral test dose absorption occurs and tends to be normal (*see* Table 3)

All of the foregoing applies only to "physiologic" amounts of B_{12} (up to 1 or 2 μg /day) Beyond these levels the percentage absorbed by normal individuals decreases rapidly (47)

Amount μg	*Absorption %*
0 5	90
1 0	80
2.0	40
5 0	22
50	3
1 000	1.5

As can be calculated the absolute amounts absorbed however rise from 0 45 to 15 μg This is true both in the normal subject and in the patient with pernicious anemia Minot and Murphy s success in treating patients with pernicious anemia by feeding them liver was probably due to the intake of such large unphysiologic amounts of vitamin B_1 that absorption although small percentually was quantitatively significant Absorption of these large amounts of B_1 across the intestinal mucosa does not depend on intrinsic factor and occurs by a sort of mass action effect (113) This effect occurs not only in the normal person and in the patient with pernicious anemia but also in the patient with malabsorption syndrome Recent reports suggest that in patients with pernicious anemia the threshold for such mass action absorption may vary thus a few patients with pernicious anemia in relapse respond to 5 μg B_{12} by mouth daily (37) many to as little as 15 to 50 μg (37) and all to 150 μg (17) Patients given huge (1 000–9 000 μg) single doses e g once a week regularly showed absorption of therapeutically effective amounts (31 107)

It is obvious then that the absorption pattern of orally administered B_{12} depends on the dosage In physiologic amounts absorption occurs in stoichiometric relation to the amount of gastric intrinsic factor (4) and cannot occur in its absence this is the normal pattern of absorption In larger dosages B_1 passes across the intestinal wall independent of the presence or absence of intrinsic factor

The advent of crystalline preparations and solutions of B_1 has pro

ABSORPTION

Intestinal Absorption

When B_{12} is taken by mouth by the normal individual absorption occurs across the intestinal wall It has been shown that intrinsic factor *produced in man in the corpus of the stomach is essential* for normal absorption of physiologic amounts of B_{12} The actual absorption occurs not in the stomach but as with other nutrients and vitamins in the small intestine and, specifically, in the duodenum and upper jejunum (25) It is uncertain if any significant absorption occurs in the rest of the small intestine or in the colon Lack of colonic absorption is further confirmed by the observation that the stools of patients with pernicious anemia in relapse (not to say those of the normal individual) contain significant amounts of B_{12} amounts so large that could they be absorbed they would produce remission (10 23)

These facts concerning normal B_1 absorption have been confirmed by radioactive technics The nature of intrinsic factor is not known That in man it is produced exclusively in the corpus of the stomach *is fully established* (25 52) *It is thought to be a mucopolysaccharide* which possibly binds B_1 for a time then releases it to the mucosal cells for absorption (25 126 129) Not all substances which bind B_1 however *can then release it* Most *of the binding substances are not* intrinsic factor For example serum globulin certain bacteria (22 33) and the Taenia Diphyllobothrium latum (97) may bind ingested B_1 When the bacteria or Taenia have bound the vitamin in competition with intrinsic factor they do not release it for absorption and thus lead to B_{12} deficient megaloblastic anemias

Even in the presence of normal amounts of intrinsic factor however and in the absence of competitive binding agents other abnormalities may prevent normal absorption of ingested B_1 This is the case in malabsorption syndrome where the defect in absorption of B_1 *is merely part of a more generalized absorption defect* and in which the addition of intrinsic factor to the oral dose of B_1 does not enhance absorption (99)

A patient may lack intrinsic factor because of a congenital defect (121) or of a heredofamilial disorder of the secreting glands of the corpus of the stomach (pernicious anemia) or of surgical ablation of these glands (total gastrectomy) In such a patient it is the lack of intrinsic factor which by causing prolonged *malabsorption of ingested*

in plasma protein which causes B_{12} depletion in the blood but malabsorption of the vitamin itself. Under certain abnormal conditions such as chronic myelocytic leukemia the ability of the blood proteins to bind B_{12} increases markedly, so that both serum levels and binding capacity reach extremely high levels. We are not personally aware of any disorder in which binding capacity for B_{12} is abnormally low although theoretically such a situation may exist, and 1 such case has been reported (60).

CHANGES WITH AGE.—The serum B_{12} level tends to fall with advancing age (12). This decrease, although statistically significant, is apparently without clinical importance since even the lower levels remain entirely within the normal range. The mechanism and significance of the decrease are not known; there is some indication, however, that with advancing age B_{12} absorption may sometimes be impaired (48).

CHANGES IN PREGNANCY.—As pregnancy progresses the serum B_{12} level falls (13, 57) but again the levels remain within the normal range. The serum B_{12} (cord blood) of newborn infants is statistically higher than that of the mothers and of normal adults (14). Apparently the fetus obtains its tissue (and therefore serum) B_{12} at the mother's expense without, however, decreasing her stores to abnormal levels. Pernicious anemia of pregnancy is generally not due to B_{12} deficiency; the mother's tissues (as measured by the serum levels) are not depleted of B_{12} nor does the disorder usually respond to B_{12} therapy. However, occasional exceptions have been reported (28, 70). The megaloblastic erythropoiesis of this disorder is due to other causes and responds preferentially to folic acid.

CHANGES IN CHRONIC MYELOCYTIC LEUKEMIA.—In some untreated cases of this disorder and in cases of acute leukemias the serum level of B_{12} is abnormally high (1,000–12,000 $\mu\mu g$/ml.) (5, 6, 58, 83). The increase is due chiefly to an increase in the protein-bound B_{12}. In chronic myelocytic leukemia it has been shown that the B_{12} is bound almost entirely to α_1 globulin (84 per cent) with 15 per cent being bound to α globulin (6, 58). The ability of such blood to bind additional B_{12} despite the already high B_{12} content differs from that of normal serum, which binds strictly limited amounts of B_{12} (9, 86, 90, 102). Since no quantitative or electrophoretic difference between the proteins of such leukemic blood and those of normal blood have been demonstrated, a qualitative difference of the α_1 globulin may be inferred.

vided a method of by passing normal intestinal absorption by the simple expedient of injecting B_{12} solution or liver extract instead of giving B_1 by mouth In pernicious anemia small injections (1–2 μg /day) of crystalline B_{12} have the same effect as large (150 μg /day) oral doses (20 32)

Absorption by Other Routes

The absorption of B_{12} by still other routes has been demonstrated under experimental conditions Such routes include the nasal (91) sublingual (19) and subcutaneous tissues

TRANSPORT

In the normal individual, B_{12} is present in the plasma partly in a free or unbound form (directly available to the assay microorganism) and partly in a protein bound form (available for assay only after heating which separates it from the protein) The total serum B_{12} amounts to 200 to 900 $\mu\mu$g per milliliter * The free serum B_{12} ranges between 0 and 200 $\mu\mu$g per milliliter and constitutes 5 to 30 per cent of the total The difference between total and free serum B_1 is the bound B_{12} (i e 200–700 $\mu\mu$g /ml) The bound B_1 is normally carried by the globulins largely by α_1 globulin (66 per cent) in part by α globulin (20 per cent) and some by β globulin (12 per cent) (58) Albumin neither carries nor binds B_1 (102) although in the presence of excessive amounts of B_{12} *in vitro* nonspecific binding of some of the excess may occur (9) Binding to the α globulins is generally accepted but some also maintain that there is nonspecific binding to and transport by the γ globulins (64) In the patient with pernicious anemia or with other B_1 deficiency disease the serum level approaches zero (50 $\mu\mu$g /ml) and whatever amount of B_1 is present is globulin bound

The B_1 in normal plasma although largely bound to protein does not completely saturate the proteins to which it is bound i e they can bind additional amounts of B_{12} This binding power of human blood has been measured by microbiologic technics and has been found to be of the order of 700 $\mu\mu$g of B_1 per milliliter (9 67 86 106) both in the normal individual and in the patient with pernicious anemia In the latter therefore it is not some abnormality of or lack

* 1 $\mu\mu g = 1 \times 10^{-12}$ Gm = 0 000 000 000 001 Gm

tient with leukemia as in the normal subject These results conform with the abnormal ability of leukemic blood to bind B_{12}

The abnormality was further demonstrated by repeating the experiment in normal individuals after incubating the radioactive B_{12} *in vitro* with a solution of human serum albumin and with serum ob

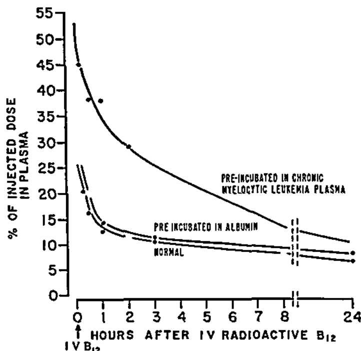


FIG 2—Effect of incubating vitamin B_{12} with albumin (no binding) and with leukemic plasma (binding of B_{12}) before administration of $Co^{60}B_{12}$ intravenously to a normal subject

tained from the leukemic patient with high serum levels of B_{12} Whereas the serum of the leukemic patient bound B_{12} *in vitro* the albumin showed no such binding (Fig 2)

With treatment the serum levels of B_{12} in patients with chronic myelocytic leukemia drop toward normal (6 83)

The source of the increased amounts of protein bound B_{12} in the serum of these patients is not known Presumably both ingested and tissue stored B_{12} becomes bound in this way

CHANGES IN LIVER DISEASE—Hepatic localization of B_{12} is demon

Similar results were obtained by measuring the disappearance of radioactivity after intravenous injection of a tracer dose In the normal subject, after rapid injection of physiologic (0 5 μg) or small (1–4 μg) amounts of radioactive B_{12} the level of radioactivity in the blood falls rapidly (78 90) (Fig 1) within 2 minutes less than 50 per cent is present in the plasma within 1 hour less than 15 per cent after 8 hours less than 5 per cent Of the remaining radioactivity less

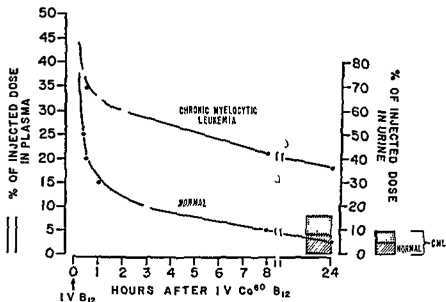


Fig 1—Radioactivity in plasma and urine after intravenous administration of $Co^{60}B_{12}$ to a normal subject and to a patient with chronic myelocytic leukemia

than 10 per cent of the dose appears in the first 24 hour urine and none thereafter the only site of localization demonstrable by *in vivo* counting technics is the liver which shows immediate uptake and a slow progressive rise (*see* Fig 3) In a patient with untreated chronic myelocytic leukemia and high serum levels of B_{12} (Fig 1) the rate of disappearance of radioactivity was significantly slower after 2 minutes 55 per cent remained in the plasma after 1 hour 32 per cent after 8 hours 22 per cent After 24 hours when the plasma of the normal subject showed no appreciable radioactivity, the leukemic patient still retained 18 per cent of the injected dose The radioactivity in the urine and the localization in the liver was the same in the pa

B_1 megaloblastic anemias may be divided into those with low and those with normal levels It is a reasonable inference that the cause of the megaloblastic anemias with normal serum (and therefore tissue) levels is not tissue depletion of B_1 The megaloblastic anemias with low serum levels of B_1 are in general due primarily to tissue depletion of B_1, and respond to B_{12} therapy

Pernicious Anemia—The serum B_1 is so low (< 50 μμg/ml) as to approach zero (67 84) Parenteral administration of liver extract or vitamin B_1 effects the return of the serum level to normal Treatment with sufficiently large oral doses is also successful in all respects—hematologic clinical and neurologic—including return of the serum level to normal (20 107)

Malabsorption Syndrome—In some patients the serum B_1 level is normal in others it is low (< 100 μμg/ml) but rarely to the degree found in pernicious anemia

Total Gastrectomy—Approximately 2 to 4 years after total gastrectomy megaloblastic anemia may occur identical in all clinical and pathogenetic respects with pernicious anemia When this occurs the serum levels of B_1 are as low as in addisonian pernicious anemia

SUMMARY

Vitamin B_1 is normally present in the serum in a "free" form and in a form bound to the serum α globulins It is carried from the sites of absorption in the small intestine to the tissues where it is utilized and stored In certain disorders e g chronic myelocytic leukemia abnormal globulins may be present in the blood which both carry and can bind abnormally large amounts of B_1 In other disorders with protein abnormalities (myeloma sarcoidosis chronic lymphocytic leukemia) we have found no such increase in binding capacity In certain forms of injury to the hepatic cells the serum B_1 level may be high because of its liberation from these cells but the ability of the serum to bind added B_{12} is not increased In the megaloblastic anemias due to B_1 deficiency the serum B_{12} level is extremely low reflecting the low tissue level

DISTRIBUTION STORAGE AND EXCRETION

It has long been known that liver obtained from a patient dead of pernicious anemia or of certain related disorders is incapable of caus

strated not only by the data of the experiments just described (Fig 3) but also by data obtained by the Glass hepatic uptake technic and by the well known fact that healthy liver contains huge amounts of B_1 (112 115 124) The B_1 is contained within the hepatic cells where according to some it is bound to a protein which resembles the β globulin of serum (102 103) It has been shown that the acutely damaged liver of an animal given injections of carbon tetrachloride shows markedly impaired ability to fix B_1 (132)

Related observations of a rise in serum B_{12} have been made in pa-

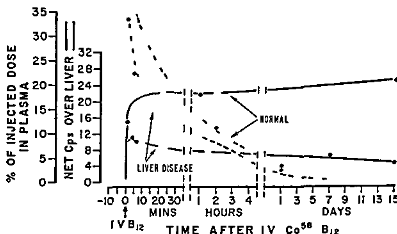


FIG 3—Radioactivity in plasma and liver after intravenous administration of $Co^{58}B_{12}$ in a normal subject and in a patient with liver disease

tients with early acute hepatitis as the hepatitis subsides the B_{12} levels return to normal (106) It has therefore been suggested that damage to the hepatic cells which normally store B_1 causes its release into the blood stream (62 106)

Intravenous injection of physiologic amounts (0 5 μg) of radioactive B_1 into patients with hepatic disease have also shown that such livers take up less than normal amounts of the vitamin (*see* Fig 3) and that the serum level of radioactivity is lower at any time than in the normal subject In other words in such patients the injected B_1 remains less readily in the plasma and cannot be accepted in normal fashion by the hepatic cells The reasons for these events are being explored

CHANGES IN MEGALOBLASTIC ANEMIAS—With regard to the serum

radioactive B_{12} show the greatest amount of B_1 to be localized within the liver (50%) with lesser amounts in the kidney, heart pituitary and other organs (personal observations)

The B_1 in the body is in a state of flux If for example in the Schilling urinary excretion test the oral test dose of radioactive B_1 is not accompanied by injection of nonradioactive B_1 urinary radio activity cannot be demonstrated but if 1 000 μg of nonradioactive B_1 is given parenterally at the same time some 8 to 40 per cent of the oral dose appears in the urine within 24 hours However if the ad ministration of the nonradioactive B_1 is delayed for 24 hours after ingestion of the radioactive dose some radioactivity (one half or one third of that in the first experiment) is found in the following 24 hours Apparently dilution of the body radioactivity with additional B_1 allows in the normal course of its utilization transport, and storage some radioactivity to be released from the stores to circulate in the blood and to be excreted If the parenteral dose is delayed for 48 hours after the oral dose there is still excretion in some cases but of even smaller amounts (79)

The excretion pattern is quite definite The normal individual daily ingests 5 to 15 μg in his food (104) and excretes 0 2 μg in his urine (84) and some 10 μg in his stool (23) The B_{12} in the urine is thought to be entirely endogenous some of that in the stool represents un absorbed B_1 and some is formed *de novo* by bacteria in the colon When the normal subject ingests a test dose of 0 5 μg of radioactive B_{12} he thus excretes 0 to 42 per cent in his stool (presumably un absorbed) and none in the urine (that absorbed is distributed to the tissues) As the amounts taken by mouth are increased more and more remains unabsorbed

The normal individual given a dose of B_1 parenterally excretes none in the stool and all above approximately 500 μg (31) in the urine within 24 hours Similar data obtained in animal experiments confirm that parenterally administered B_1 appears only in the tissues and in the urine but not in the stool whereas ingested B_1 appears mainly in tissues and stool and only a small amount in urine

In the normal subject and in the patient with pernicious anemia the amounts of B_1 in the feces are large Apparently the B_1 is formed by colon bacteria and is not available to the body because it is not absorbed (8 23 42) The patient with an absorptive defect for B_1 loses nearly all the ingested dietary B_{12} in the stool the urine con taining only minute amounts

ing remission of pernicious anemia in relapse in contrast to liver from a patient dead of other causes (43 108) Microbiologic assay of the liver of a patient with pernicious anemia in relapse shows absence of B_1 (43 123) The chief source of the antipernicious anemia factor has always been liver extract Newer studies have confirmed if such con firmation were needed the importance of the liver as the storage depot of B_1

Organ analyses of animals given B_1 show most of it to be lodged in the liver and kidneys (80 115) In the normal human subject orally administered radioactive B_1 rapidly appears in the liver and radioactivity persists for more than a year (117) After intra venous administration of radioactive B_{12} to a normal subject there is prompt uptake by the liver and persistent localization there of radio activity (*see* Fig 3) such localization is not demonstrated by this technic in other organs When the patient with pernicious anemia is given radioactive B_1 by mouth together with a potent source of in trinsic factor radioactivity also appears in the liver within 5 to 7 days and persists In patients with chronic myelocytic leukemia (75) or other lymphomas radioactivity appears in the liver immediately after intravenous administration and increases in amount in the course of the next 3 weeks (75 personal observations) In the patient with hepato cellular disease radioactivity still appears promptly in the liver but in smaller amounts than in the normal subject and it tends to fall off rather than to rise (*see* Fig 3) *In vivo* counting over other sites—heart spleen thigh muscles—fails to show within the limits of the method localization of radioactivity There is thus no question that the liver cell is the prime site of localization of vitamin B_1 in the body

However B_1 is also present in other tissues (112 115) and is ex creted from the body in definite patterns Intravenously injected radio active B_1 appears in minute amounts in the cerebrospinal fluid (84) but none has been found in ascitic fluid (78 personal observations) It remains in the plasma for a period of time which depends chiefly it seems on the ability of the plasma proteins to bind it (*see* Figs 1 and 2) It normally appears in the urine in small amounts less than 10 per cent of the injected dose in 24 hours Since only 10 to 15 per cent can be accounted for by the amounts in plasma and in urine the re maining 85 per cent must be within the body and largely within the liver Clinical knowledge however compels the conclusion that B_1 is present in all body tissues, notably in the cells of the bone marrow and the nervous system Postmortem organ analyses of patients given

essary for the production of cells normal in size appearance and function A tissue deficiency of B_{12} or folic acid (or theoretically of any of the other substances) results in abnormal nucleoprotein synthesis with a series of distinct abnormalities in the cells The most marked abnormality is seen at the hematopoietic centers where normal normoblastic erythropoiesis is replaced by megaloblastic erythropoiesis

TABLE 4—PATHOGENETIC CLASSIFICATION OF MEGALOBLASTIC ANEMIAS

CAUSE	TYPE OF ANEMIA
Tissue deficiency of B_{12}	
B_{12} lack in diet	Nutritional macrocytic anemia
Malabsorption of B_{12}	
Absence of intrinsic factor	Pernicious anemia total gastrectomy congenital in children
Multiple defects of intestinal absorption	Primary and secondary malabsorption syndromes
Bacterial utilization of B_{12}	Intestinal blind loop syndrome
Parasitic competition for B_{12}	Diphyllobothrium latum anemia
Impaired transport of B_{12}	Defective α-globulins megaloblastic anemia (60)
Poor utilization of B_{12}	Megaloblastic anemia in liver disease (?)
Destruction of B_{12}	None known
Tissue deficiency of folic acid	Nutritional macrocytic anemia primary malabsorption syndrome intestinal blind loop syndrome pernicious anemia of pregnancy megaloblastic anemia of infancy megaloblastic anemia due to anticonvulsant drugs
Tissue deficiency of ascorbic acid	Megaloblastic anemia of infancy

poiesis Related defects occur in other hematopoietic cells (abnormally large granulocytes abnormally maturating megakaryocytes) and in other tissues notably epithelial cells (50) and nerve cells (94) The clinical result of B_{12} deficiency i e of tissue depletion is therefore a systemic disease involving the gastrointestinal mucosa and the nervous and hematopoietic systems The systemic nature of the clinical disease is to be expected whatever the cause of the tissue deficiency lack of intrinsic factor (pernicious anemia, total gastrectomy) malabsorption of B_{12} (malabsorption syndrome) competition for B_{12} (Diphyllobothrium latum infestation blind loops) or dietary lack of B_{12} (nutritional macrocytic anemia) Too the same clinical end results—the systemic picture of megaloblastic anemias—may result from tissue depletion of folic acid ascorbic acid, lack of carbon

ROLE IN CELLULAR METABOLISM

Multiple metabolic functions have been discovered for vitamin B_1 including a role in carbohydrate (29) and fat metabolism (65 69 94 98) Another role is in the synthesis of the nucleoproteins of the tissue cell. The mechanism of its action is not known but it has been shown that it plays a part in the methylation of homocysteine the formation of methionine desoxyribosides and purines the reduction of thiol groups (glutathione homocystine and perhaps coenzyme A) and presumably in still other as yet unclarified reactions (2) In the

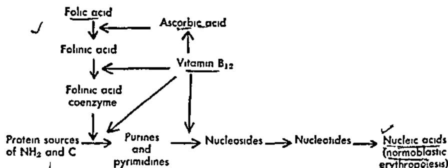


Fig 4—Role of vitamin B_{12} in nucleoprotein metabolism theoretic schema of some interrelations of vitamin B_{12} folic acid and ascorbic acid (Adapted from Girdwood (40) and Mueller and Will (89))

intermediary metabolism of nucleic acid precursors B_{12} is associated with folic, folinic and ascorbic acids Figure 4 presents a schema embodying the current concepts of the formation of normal nucleo protein (40 93)

Vitamin B_1 is thus one of many substances which although essen tial to the normal formation of the tissue cell, are not synthesized by vertebrates The schema suggests that several substances are impor tant and necessary for the normal development of the tissue cells proteins folic and ascorbic acids B_1 and presumably other sub stances not shown in Figure 4

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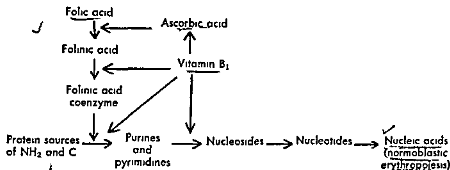


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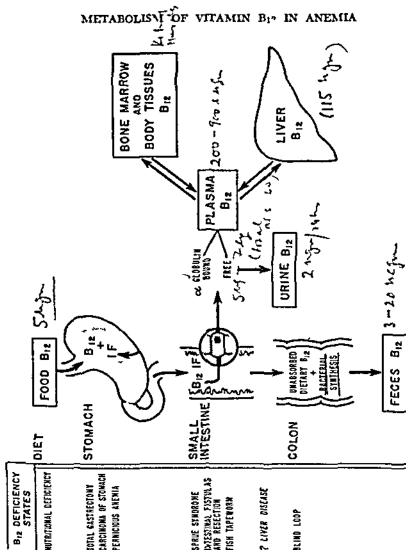


FIG 5 —Absorption transport and storage of vitamin B_{12} schema of the pathogenesis of vitamin B_{12} deficiency states

Nutritional Macrocytic Anemia

The megaloblastic erythropoiesis in this disease is probably due to multiple deficiencies vitamin B_{12} protein folic acid and other substances However in some cases the B_{12} deficiency is of prime importance Thus strict vegetarians ("vegans") were found to have low serum levels of B_{12} (130) other patients with dietary fads who develop megaloblastic anemia on a nutritional basis have shown low serum levels despite normal intestinal absorption of B_{12} (105) In such pa

donors (proteins, amino acids) and perhaps other factors Table 4 gives a pathogenetic classification of the megaloblastic anemias on this basis Clinically of course there are differences between the syndromes caused primarily by deficiency of B_1 and those due to deficiency of the other substances notably folic acid Thus involvement of the central nervous system is much more common in the B_{12} deficiency anemias and especially in pernicious anemia than in folic acid deficiencies such as certain cases of sprue or in nutritional macrocytic anemia Nevertheless, subacute combined degeneration has been described in sprue (133) and in nutritional macrocytic anemia Clinically an excess of folic acid in the presence of B_1 deficiency (pernicious) anemia may lead to neurologic involvement previously absent, despite hematologic remission apparently the cause is a reciprocal relation between B_{12} and folic acid so that in a deficiency of one of them administration of the other aggravates the deficiency (55) Vitamin B_1 seems to be of special importance to the well being of the nerve cell (94) administration of folic acid by furthering the reactions shown in Figure 4 results in even further depletion of the remaining B_1 in the nerve cells with disastrous results

The data on B_1 absorption transport and storage suggest that its serum levels are merely an index of its tissue stores (85) Observation of patients with pernicious anemia accidentally or deliberately allowed to relapse by withholding therapy confirms that the tissue stores at the height of an induced remission are adequate to supply the patient for several years Patients with total gastrectomy similarly do not have megaloblastic anemia until after 2 to 4 years During this time determination of the serum B_1 levels by giving a clue to depletion of tissue stores may give notice of impending relapse Patients treated with oral B_1 without intrinsic factor are slow to reach normal serum B_1 levels (20) despite complete hematologic and neurologic remission This suggests that in such cases too the tissue content is precariously low and that relapse may occur swiftly if treatment is discontinued

ROLE IN MEGALOBLASTIC ANEMIAS

From the discussion of the absorption and utilization of B_1 a hypothetic diagram can be prepared listing theoretic pathogeneses for most of the B_1 deficiency megaloblastic anemias (Fig 5) However not all megaloblastic anemias are attributable to deficiency of B_1 (*see* Table 4)

plete remission in all cases) In our own experience the serum B_{12} levels approached normal in most of these cases (20) others also have found normal serum levels of B_1 (107) Orthodox treatment circumvents the intestinal barrier to B_{12} absorption by parenteral administration of B_1 or liver extract (a source of B_1) remission is complete the serum B_{12} level returns to normal and the tissues become saturated Progress of the central nervous system lesions is halted and improvement generally occurs although often to a limited degree)

Total Gastrectomy

This operation by removing the glands producing intrinsic factor (100) causes an acquired inability to absorb B_1 comparable to the heredofamilial defect causing pernicious anemia After 2 years or more a clinical picture develops identical in all respects with that of pernicious anemia (114) The results of B_1 absorption tests are identical with those in pernicious anemia (49 122) (*see* Table 3) the serum levels of B_1 are low (*see* Table 1) and treatment is the same as for pernicious anemia Preventive treatment consisting of parenteral administration of B_{12} at stated intervals after the operation is now recommended for all patients who have had a total gastrectomy)It prevents depletion of tissues stores of B_{12} and thus the development of the pernicious anemia picture

If gastrectomy is not truly total the glands which produce intrinsic factor may not be entirely removed and B_1 deficiency does not develop Subtotal gastrectomy is thus inconstantly followed by a B_1 deficiency (3) It has been said that the leaving behind at surgery of a 1 to 2 cm collar of the gastric fundus may be enough to prevent the development of postgastrectomy pernicious anemia (53)

Congenital Absence of Intrinsic Factor

Some workers have assumed that in certain children with the hematologic picture of pernicious anemia there was a congenital defect of the gastric mucosa which led to congenital lack of intrinsic factor and thence to pernicious anemia (7 89 121) This situation in our opinion is exceedingly rare

Posterolateral Sclerosis without Anemia

In certain cases posterolateral sclerosis due to B_{12} deficiency occurs without or before development of the hematologic changes of pernicious

tients, B_1 therapy results in remission (105) but generally the use of full diets supplemented by *multiple vitamins is recommended*

Penicillin has also produced remissions in such patients (40) Presumably the antibiotic eliminates bacteria which have competed successfully for ingested B_1 thus allowing normal B_{12} absorption and leading to subsidence of the megaloblastic anemia

Pernicious Anemia

This disorder, the prototype of the entire group of megaloblastic anemias is today known to be the end result of a heredofamilial defect of the gastric glands as a result of which the gastric fundus sooner or later fails to produce intrinsic factor Since intrinsic factor is necessary for the normal transport of dietary B_{12} across the intestinal mucosa *progressive depletion of the tissue store of B_{12} develops* with resultant abnormalities of nucleoprotein synthesis in the gastrointestinal tract and nervous and hematopoietic systems The diagnosis is suggested by the finding of pancytopenia with megaloblastic anemia in the presence of achylia it is strengthened when other causes of megaloblastic anemia are eliminated and it is established beyond doubt by demonstrating the intestinal inability to absorb physiologic amounts of B_{12} *in the absence of intrinsic factor* The serum and urinary B_{12} levels are extremely low approaching zero)

The pathogenesis of pernicious anemia already discussed is the *chronic inability to absorb dietary B_1 because of the lack of gastric* intrinsic factor Certain recent observations have suggested however that an abnormal intestinal flora may contribute to the development of pernicious anemia remission after antibiotic therapy *has supported* this suggestion (68) However such observations are not fundamental to the basic concept of the disorder and such therapy is only of experimental interest

Folic acid therapy for pernicious anemia in relapse temporarily causes hematologic remission but may precipitate neurologic relapse Treatment with small amounts of B_{12} by mouth (5–50 μg/day) without added intrinsic factor is not consistently effective apparently there is mass action transport of part of the B_{12} across the intestinal mucosa in some cases (37) Oral treatment with small amounts of B_{12} together with intrinsic factor is probably always effective provided the intrinsic factor is potent Large amounts of B_1 by mouth (150 μg/day to 1 000 μg/week) without intrinsic factor by forcing absorption independent of the intrinsic factor mechanism causes com

show that the abnormal erythropoiesis responds in some cases only to B_{12} in others only to folic acid

The defect in the intestinal absorption of B_1 in this syndrome differs from the defect in pernicious anemia in the former the defect is not related to intrinsic factor (*see* Table 3) In the patient with sprue who fails to absorb B_1 in oral test doses intrinsic factor has no corrective action This fact affords an added tool in the diagnosis of megaloblastic anemias (99)

Occasionally a patient with primary sprue may show the same neurologic abnormalities as are found in pernicious anemia (133) Judging by the disastrous results of using folic acid in patients with pernicious anemia and posterolateral sclerosis the assumption that in such cases of sprue it is the depletion of B_1 which causes the central nervous system lesions seems reasonable

On the basis of the B_1 serum levels in primary sprue the syndrome can be divided into 2 groups that in which the serum level is low and that in which it is normal (77 85 87) When low the serum level ranges below 100 $\mu\mu$g per milliliter but rarely approaches the very low values seen in pernicious anemia Again the patient with sprue whose serum (and therefore tissue) levels of B_{12} are low will probably respond to B_1 therapy whereas the patient with normal serum B_1 levels responds better or perhaps exclusively to folic acid (85)

Intestinal Blind Loop Syndrome (53 125 127 128)

Megaloblastic anemia may occur in association with intestinal resections and anastomoses which create a blind loop of intestine It may also occur in cases of intestinal diverticulosis (22) a sort of multiplicity of small blind loops The essential pathogenetic factor seems to be stasis of intestinal contents and overgrowth of bacteria within the blind loops Absorption studies in such patients have demonstrated impaired intestinal absorption of B_1 and the megaloblastic anemia has been attributed to B_{12} depletion in the bone marrow

The absorption pattern of ingested B_{12} is unique (*see* Table 3) The patient fails to absorb the test dose of radioactive B_1 and intrinsic factor does not improve the absorption However in some patients given a course of Aureomycin by mouth intestinal absorption of orally administered B_{12} becomes normal (53) That this is not due to a direct effect of the antibiotic but to its antibacterial action is suggested by similar effects of orally administered gentian violet (131)

These data compel the conclusion that in such patients the static

anemia Not all cases of subacute combined degeneration are due to the deficiency some may be caused by lead intoxication some by syphilis and some by vascular abnormalities Posterolateral sclerosis related to B_{12} deficiency may occur not only with pernicious anemia itself *but with other B_{12} deficiency states* malabsorption syndrome (133) nutritional macrocytic anemia blind intestinal loops (125)

Patients with posterolateral sclerosis in pernicious anemia (whether the anemia is present or not) show profound reduction of the B_1 serum levels (87) Radioactive technics show the same pattern of failure of absorption as in pernicious anemia intrinsic factor increases the intestinal absorption of the test doses of B_{12} toward normal (1)

Such cases of posterolateral sclerosis without anemia must therefore be considered as B_{12} deficiency syndromes in which the depletion of tissue B_1 primarily affects for reasons which are quite obscure, not the hematopoietic tissues but the axons Intensive saturation of the body (and therefore the nerve cell) with B_1 by parenteral administration of large doses of crystalline B_{12} prevents further neural damage and may result in considerable remission of neurologic symptoms

The adverse effects of administration of folic acid in such cases is proof that folic acid is not the cause of the neural damage It is postulated (Fig 4) that an excess of folic acid in a patient depleted of tissue B_1 forces the utilization of still more B_1 and thus increases the neural damage (93)

Primary Malabsorption Syndrome

A defect in the intestinal absorption of B_1 has been demonstrated in most but not in all cases of primary malabsorption syndrome in nontropical sprue tropical sprue and idiopathic steatorrhea (99) as well as a defect in folic acid absorption (45) However these are merely part of a large group of defects of intestinal absorption including defective absorption of iron vitamins A and K fats and other substances The resulting clinical and hematologic pictures therefore vary from case to case depending in part on the predominant absorptive defect(s) (36)

Hematologically the characteristic picture of the malabsorption syndrome is a macrocytic anemia with megaloblastic or intermediate megaloblastic *erythropoiesis in the bone marrow* (36) The pathogenesis of the abnormal erythropoiesis as between B_1 deficiency and folic acid deficiency is not clarified It is likely that both deficiencies as well as iron and protein deficiencies are important Therapeutic studies

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The pathogenesis of the megaloblastic anemia of D latum infestation is competition between parasite and host for ingested B_{12} in which the worm successfully competes against the host's intrinsic factor by binding most of the ingested B_{12} (17) The patient therefore does not absorb B_{12} and tissue deficiency of B_{12} results in megaloblastic anemia The serum B_{12} levels in these patients are extremely low (96 97) The radioactive oral absorption tests (*see* Table 3) reveal poor absorption of the test dose and most of the ingested radioactivity *is found in the worm (18 96) Intrinsic factor does not overcome the* malabsorption of the test dose The presence of free hydrochloric acid also suggests that pernicious anemia is not the correct diagnosis and the finding and expulsion of the worm are followed by remission of the anemia

Pernicious Anemia of Pregnancy

Although the serum levels and tissue content of B_{12} fall during pregnancy even the lower levels are within the normal range (13) Pernicious anemia of pregnancy would therefore not be expected to be due to tissue depletion of B_{12} and clinical observations have long confirmed this fact. In this disorder the serum levels of B_{12} are not low and, with rare exceptions (28 70) the disorder responds not to B_{12} therapy but to folic acid administration

The pathogenesis of pernicious anemia of pregnancy must be sought elsewhere in the schema (*see* Fig 4) perhaps in some unexplained deficiency of folic acid (coenzyme) (70 93 125)

Pernicious anemia of pregnancy is not a B_{12} deficiency anemia

Megaloblastic Anemia of Infancy

The pathogenesis of the megaloblastic anemia which sometimes occurs in infants is also not to be found in study of B_{12} depletion A megaloblastic anemia which resembles that of infancy can be produced in monkeys maintained on a milk diet deficient in ascorbic acid (71 72) This anemia cannot be prevented or eliminated by administration of B_{12} but can be successfully treated with ascorbic acid folic acid, or folinic acid It is suggested that a deficiency of ascorbic acid interferes with the metabolism of folic acid and thus leads to megaloblastic anemia (*see* Fig 4)

Interference with folic acid metabolism has been postulated as the cause of megaloblastic anemia in infants in the course of infections

bacteria within the blind loops compete in some manner with the host for the ingested B_{12} The manner of competition is not clear, it may be by a distant "toxic" action of the bacteria on the ingested B_1 on the intrinsic factor or on the B_1 -intrinsic factor complex or by a toxic effect on the upper intestinal mucosa or by a direct action in which the bacteria actually bind the B_{12} or utilize it and folic acid so that they become unavailable for absorption (125 128) Development of the B_{12} deficient megaloblastic anemia in experimental animals depends on special features the blind loop must be at the jejunum not at the ileum (128) for megaloblastic anemia to develop In such animals the resulting anemia can be cured by the parenteral administration of B_{1} but it can as well be corrected by sterilizing the bowel by means of antibiotics or by correcting the loop surgically (127 128) Sometimes only surgical correction effects remission

Depletion of tissue B_1 can thus be produced by intestinal bacteria which compete with the body for the ingested B_1 Such bacterial action has also been suggested as part of the pathogenesis of the megaloblastic anemia of nutritional deficiencies (40) and even of pernicious anemia itself (68) The intestinal flora of patients with addisonian pernicious anemia is known to be abnormal partial remissions in such patients by antibiotics have been reported and attributed to their bacteriostatic action (68) Whether this mechanism plays an important role in the pathogenesis of pernicious anemia is questionable, but variations in flora may be important in accounting for the spontaneous remissions which occur in untreated cases

Diphyllobothrium Latum Anemia

Some patients who harbor D latum have coincidental addisonian pernicious anemia others have a megaloblastic anemia hematologically identical with pernicious anemia which is the direct result of the parasitic infestation The latter have a normal content of gastric hydrochloric acid and their gastric juice contains intrinsic factor The worm must be located in the upper jejunum (16) If the worm is expelled from the body spontaneous remission of the megaloblastic anemia occurs If the expelled worm is ground up and the resulting material ingested by or injected into a patient with addisonian pernicious anemia, remission is produced (17) Finally analysis of the expelled worm for its B_{12} content shows large amounts of this substance (18)

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(73) It has been treated successfully with combined ascorbic acid folic acid and B_{12} therapy

Megaloblastic anemia of infancy too is not a B_{12} deficiency anemia

Megaloblastic Anemia of Hepatic Disease (92)

In many cases of chronic hepatic insufficiency the macrocytic anemia is not megaloblastic in etiology Rarely however a true megaloblastic anemia may occur in chronic liver disease Theoretically (Fig 5) such an anemia might be expected as the result of the liver s inability to store B_{12} Actually the pathogenesis of this anemia in liver disease is not at all clear

Megaloblastic Anemia Due to Drugs (46)

Megaloblastic anemia has been described following the use of primidone (Mysoline) and certain other drugs used to control epilepsy In such cases no defect in B_{1} metabolism has been found and folic acid has proved to be therapeutically effective It has been proposed that the megaloblastic abnormality is the result of competitive in hibition of an enzyme using folic acid Therefore this is not a B_{1} deficiency syndrome

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Corticosteroids and Infections*

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THE DISCOVERY OF the effect of adrenocortical steroids in altering the manifestations of a variety of clinical disorders was in common with many other important medical discoveries a clinical observation without an adequate theoretic framework for predicting the observed effects Much of the subsequent investigation of the action of adrenocortical steroids necessarily followed the same pragmatic pattern and coincidentally increasing attention was paid to mechanisms of action

The initial studies on the role of adrenocortical steroids in infectious disease were therefore undertaken pragmatically Many of the diseases in which effects of corticosteroids had already been observed were diseases of uncertain etiology in which immunologic mechanisms were presumed to play a role It was hoped without much logic that by studying the effects of adrenocortical steroids in infectious diseases in which a reasonable amount of information was available concerning etiology pathogenesis and mechanisms of resistance insight into some of the mechanisms of action of these steroids would be obtained In addition there were many indications of a role of adrenocortical steroids in mechanisms of resistance The observations on this aspect had for the most part been made before the discovery of the clinical effects of large doses of corticosteroids in various inflammatory diseases and were not as potent a stimulus to investigation as the observed clinical effects

In the initial studies adrenocorticotropin was given to carefully

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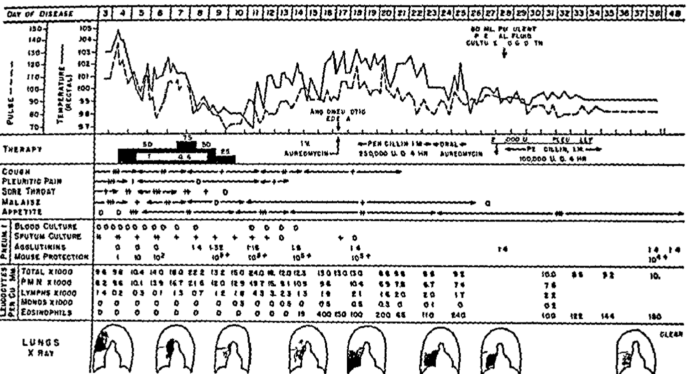


FIG 2—ACTH therapy in 48 year old man with pneumococcal pneumonia complicated by empyema Administration of ACTH resulted in gradual but progressive improvement in patient s symptoms and general appearance but despite continued therapy pneumococci persisted in sputum new lung areas became involved and empyema developed Cessation of ACTH and institution of antibiotic therapy led to gradual improvement Type specific antibodies developed by end of first week of illness and increased while large doses of ACTH were being given (From Kass *et al* (84))

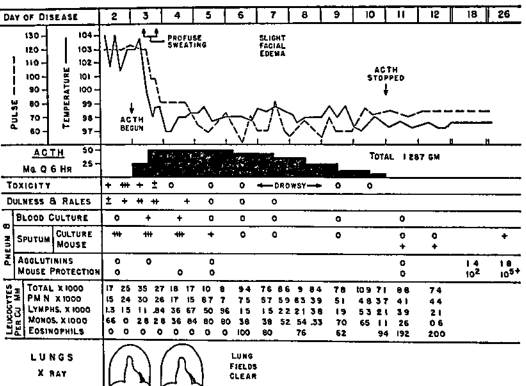


FIG 1 —ACTH therapy without antibiotics in a 16 year old boy with pneumococcal pneumonia Administration of ACTH resulted in prompt defervescence with rapid clearing of the signs and symptoms of pulmonary infiltration pneumococci nevertheless persisted in sputum after temperature fall Blood cultures showed no growth of organisms at start of treatment but yielded pneumococcus type 8 after 12 and 36 hours of therapy although patient looked and felt well Type specific agglutinins and mouse protective antibodies were not demonstrable until 2 weeks later (From Kass *et al* (84))

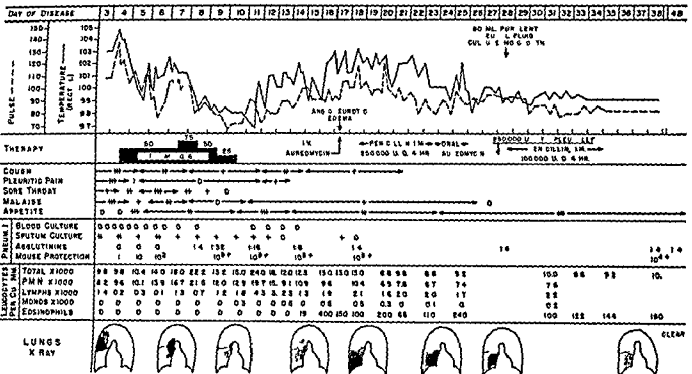


FIG 2—ACTH therapy in 48 year old man with pneumococcal pneumonia complicated by empyema Administration of ACTH resulted in gradual but progressive improvement in patient s symptoms and general appearance but despite continued therapy pneumococci persisted in sputum new lung areas became involved and empyema developed Cessation of ACTH and institution of antibiotic therapy led to gradual improvement Type specific antibodies developed by end of first week of illness and increased while large doses of ACTH were being given (From Kass et al (81))

selected patients with lobar and viral pneumonias (43 80) Figure 1 illustrates the nature of the response of a patient with type 8 pneumococcal pneumonia This and similar observations made it clear that adrenocortical steroids could induce in patients with pneumonia striking defervescence and symptomatic relief with diminution or disappearance of malaise and anorexia These remarkable symptomatic changes occurred despite the persistence of pneumococci in the sputum the presence of bacteremia and in one instance the spread of the pneumonia to other lobes with subsequent development of empyema (Fig 2) The symptomatic changes were independent of gross changes in the antibody response and were not accompanied by reversal of metabolic patterns characteristic of pneumonia and other severe injuries that is loss of nitrogen potassium and phosphorus and retention of sodium and chloride Indeed the administration of corticotropin accentuated the metabolic changes while bringing about symptomatic relief

In subsequent years considerable clinical and laboratory data have been accumulated in an attempt to answer the most obvious questions posed by these observations Is the administration of corticosteroids to patients with infectious diseases really valuable? Are there specific areas within the broad field of infectious diseases in which they are particularly useful? Are there definable conditions under which they are not helpful or in which they may be harmful? Can the specific action of these agents be so defined that they can be used to advantage without leading to serious untoward consequences? Finally can the observed effects be utilized as a means for increasing our understanding of mechanisms of resistance to infection and perhaps of mechanisms of action of corticosteroids?

Complete and satisfactory answers to these questions are not yet possible However some of the available data as they relate to the practical clinical problem as well as to the understanding of mechanisms of resistance and of hormonal action will be summarized here Many reviews of these aspects have appeared (50 72 76 77 106 124 141)

ROLE OF ADRENOCORTICAL HORMONES IN MANAGEMENT OF INFECTIOUS DISEASES

Fundamentally two conflicting points of view have developed On the one hand there is a substantial body of evidence that corticoste

roids depress resistance to infection on the other there are potent arguments for the use of corticosteroids in the management of infections

Evidence for Depression of Host Resistance by Corticosteroids

Clinical.—In the early phases of the study of the problem corticosteroids without antibiotics were used in the treatment of such diverse infections as pneumococcal and other bacterial pneumonias tuberculosis brucellosis, typhoid fever subacute bacterial endocarditis acute streptococcosis leptospirosis peritonitis tetanus rickettsiosis poliomyelitis smallpox mumps viral hepatitis viral pneumonias infectious mononucleosis malaria, trichinosis blastomycosis and undoubtedly others (72 76 77 141) The specific etiologic agents were not always clearly definable and many of the studies consist of reports of single or small numbers of cases Nevertheless a few generalizations can be made from the observations recorded on this great variety of infections

(1) The administration of corticotropin or corticosteroids to patients with acute febrile illnesses or with severe malaise or anorexia frequently brought about prompt defervescence and amelioration of many of the distressing clinical manifestations of illness

(2) In those infections in which cultivation or demonstration of the infectious agent was possible during the course of treatment with corticosteroids alone for example in bacterial pneumonias typhoid fever tuberculosis brucellosis subacute bacterial endocarditis there was frequently evidence of increased multiplication or spread of the pathogenic agent despite the apparent amelioration of the clinical syndrome

(3) Many of the disease syndromes treated with corticosteroids are of the type in which quantitation of the etiologic agent is difficult or impossible (*e g* trichinosis viral hepatitis infectious mononucleosis) Most of these are infections in which the mortality rates are low and complications are infrequent Presumably host resistance in these diseases is high and even if significant depression of immunity were to occur detection would be difficult However carefully controlled studies of the effects of corticosteroids in infectious hepatitis have shown that although the use of corticosteroids may lead to more rapid symptomatic improvement increased appetite and more rapid fall in serum bilirubin than in untreated controls relapses occurred in

20 per cent of the treated patients and did not occur in controls The concurrent administration of human serum γ globulin did not prevent relapses in the treated patients (41 42)

Controlled studies of the effects of corticosteroids on the course of poliomyelitis (27) and of acute streptococcosis (58) have indicated no striking effect on the acute disease nor on the sequelae of these two infections the patients were neither benefited nor apparently harmed When live virus was injected therapeutically into patients with malignant disease treated with cortisone the duration of viremia and the resulting infections were only slightly higher than in similar patients not receiving cortisone (136)

Rapid subsidence of mumps orchitis following the administration of corticosteroids has been reported (110 135 137) More detailed studies have indicated that azoospermia and testicular atrophy nevertheless occurred in some of the patients so treated and that a beneficial influence of corticosteroids on testicular function after mumps orchitis must be considered unproved (89) Although it has been pointed out that those patients with mumps orchitis who became afebrile most slowly were the ones in whom late testicular complications were most likely to develop and that more vigorous treatment might have proved more effective it is just as reasonable to suppose that late azoospermia and atrophy develop only in the severe cases of mumps orchitis and that corticosteroids are of little value in such cases In a controlled study by Klemola and Somer (89) no difference in response of mumps orchitis to corticotropin or to placebo was discernible with substantial but not extremely large doses

Finally the appearance of infections either spontaneously or as aggravations of pre existing ones in patients receiving corticosteroids for treatment of other diseases, is well known Pneumonias bacteremias of various etiologies pyelonephritis staphylococcal endocarditis localized staphylococcal abscesses cellulitis peritonitis meningitis tuberculosis moniliasis histoplasmosis and other mycoses malaria and even unusual saprophytic infections have appeared in patients who were receiving adrenocortical hormones (76 77) The appearance of infections during the course of treatment with corticotropin or corticosteroids has been a major reason for temporary or permanent cessation of treatment with corticosteroids and many warnings have been issued to physicians urging *circumspection in the use of corti*costeroids particularly in disease states in which pulmonary tuberculosis might be present Haggerty and Eley (57) collected 12 fatal

instances of varicella in children who were receiving such hormones at the time of exposure to this disease

The precise magnitude of the hazard of infection in patients receiving corticosteroids is difficult to assess In many cases administration of corticosteroids has so masked the appearance of severe sepsis that the infections were recognized only at autopsy In other cases patients with fever of unknown etiology have been treated with corticosteroids on the assumption that a disease other than tuberculosis was present, only to show such progression of active tuberculosis as to make it likely that this diagnosis had been missed Undoubtedly also the magnitude of the hazard of infection varies with the group of patients under treatment It is reasonable to suppose although there is no clear supporting evidence that intercurrent infections are more likely to develop in patients receiving corticosteroids in the treatment of severe debilitating illness than in those being treated for milder self limited illnesses or for chronic illnesses such as rheumatic fever in which convalescence is prolonged but debility is not commonly a prominent feature

Thus Benedek and Montgomery (10) did not consider the incidence of infection to be unusually high in their patients and several studies have indicated that the hazard of infection is definite but not very great (53 59 98 100) On the other hand Ragan and co-workers (68 115) reported that they considered it necessary to discontinue treatment with corticosteroids permanently in 10 per cent and temporarily in another 10 per cent of their patients with rheumatoid arthritis In a Danish study moniliasis was reported to have developed in 15 among 210 patients with various primary diseases while receiving corticosteroids but the disease was relatively mild in 11 and might have gone unrecognized except for the particular interest of the observers (19) In their initial studies of patients with disseminated lupus erythematosis Soffer *et al* (133) observed infections in 43 per cent of patients but in later studies this figure had fallen to 13 per cent, probably as a result of improved methods of management, but possibly due also to the earlier recognition of milder cases of lupus erythematosus in which conceivably there was less hazard of infection

It is noteworthy that in spontaneous hypercorticoidism (Cushing s disease) infection is the leading cause of death accounting for more than half of the deaths in a large series studied by Plotz and co workers (113) this extraordinary incidence of fatal infections has not

decreased significantly since the advent of modern antibacterial therapy Some doubt has been expressed concerning the meaning of this high mortality because the incidence of infections in arthritics at autopsy is almost as great (113)

EXPERIMENTAL —The demonstration that large doses of cortisone cortisol or corticotropin generally depress resistance to infection is documented about as well as any recent scientific demonstration It is no longer profitable to list the pathogens that have been tested most of these have been listed by us elsewhere (76 77) The variety of bacterial viral fungal protozoal and even helminthic agents encompasses most of the commonly studied pathogens and a substantial number of rare esoteric and even previously unknown agents Furthermore this anaclastic* effect on resistance has been demonstrated not only in man but also in rabbits rats mice guinea pigs and monkeys Some generalizations may be drawn from the data

(1) The effect of cortisone cortisol or corticotropin in depressing resistance is variable and depends on the dose type of hormone used experimental animal and the general nature of the experimental design

(2) The depression of resistance is frequently great enough to cause the animals to succumb to infections from autochthonous saprophytes

(3) Latent infections are frequently activated An illustrative experience is that of Denny and Thomas (30) who showed that the administration of cortisone more than 3 months after the subsidence of acute streptococcal infections in rabbits led to exacerbation of the infection with death of the host animals

(4) Depression of resistance occurs whether the resistance is native as in the case of rabbits made genetically resistant to tuberculosis or acquired passively or actively by the use of vaccines sera or antibiotics (76 77)

(5) When native resistance of the host to the pathogen is already minimal as in the case of mice infected with virulent pneumococci or of guinea pigs with tubercle bacilli the effect of cortisone is difficult to measure and is necessarily limited to accelerating the progress rate of the infection

(6) The depression of resistance may be used to facilitate the isola

* Anaclastic (Greek ana back + klan to bend) suggested as a term to describe the temporary backward deviation of resistance to infection under the influence of cortisone The term has been used to describe the bending back of thin glass with a crackling sound

tion or demonstration of infectious agents For example cortisone treated monkeys are routinely used in determining the viability of poliomyelitis virus in the manufacture of vaccine Many similar uses of cortisone treated animals have been suggested (45 71 88 140) It is not clear how cortisone facilitates adaptation of a given agent to a host. Such a method of isolating an infectious agent is inherently open to the suspicion that autochthonous or latent infections may arise in the cortisone treated animal and produce pathologic changes open to misinterpretation

(7) Frequently depression of resistance to a given pathogenic agent by administration of corticosteroids has been taken as evidence that adrenocortical secretions play a special role in the pattern of resistance to the infection under study In a general sense such a point of view is undoubtedly true However the data suggest that the mechanisms of resistance depressed by corticosteroids are not specifically related to a particular infectious agent but rather are broadly non specific in their action

USE OF CORTICOSTEROIDS WITH ANTIMICROBIAL AGENTS IN INFECTION

From the foregoing, it may be anticipated that antimicrobial agents would tend to counteract the adverse effects of corticosteroids Similarly the limitations of the effects of such antimicrobial agents may be surmised A sufficient dose of antiserum or of a specific antibiotic will usually overcome the adverse effects of a given amount of corticosteroid conversely increasing the dose of corticosteroids may overcome the effect of a given dose of specific protective agent This interplay is limited by the toxicity of the contrasting substances For example the protective activity of penicillin in pneumococcal or group A streptococcal infections is diminished by the concurrent administration of cortisone but this effect of cortisone is readily overcome by increasing the dose of penicillin (45 71) However if a tetracycline or chloramphenicol is used instead of penicillin the adverse effect of cortisone is not so readily overcome (71) there are at least two reasons for this (1) the broad spectrum antibiotics are bacteriostatic and not bactericidal and (2) the toxicity of these drugs limits the increase in dosage (Fig 3)

As might be expected the host s resistance plays a large role in determining the relative responses to corticosteroids and to antibacterial drugs For example relatively small amounts of streptomycin will over

come the effects of cortisone on tuberculosis in rats or rabbits, whose native resistance is relatively high On the other hand even with large doses of streptomycin it is difficult to overcome all of the adverse effects of cortisone on tuberculosis in guinea pigs (72). Thus the ability of specific agents to overcome the adverse effects of corticosteroids in infection is a function of the host s resistance and the organism s viru

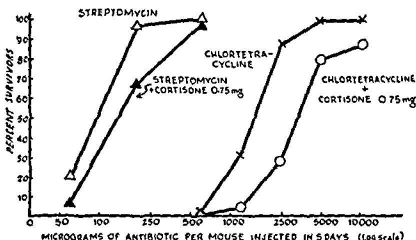


FIG 3—Effect of cortisone on therapeutic efficacy of antibiotics in mice infected with Klebsiella pneumoniae All mice were given a uniform inoculum of the organism and were treated with increasing doses of streptomycin or chlortetracycline alternate groups were given daily 0 15 mg cortisone acetate subcutaneously Cortisone decreased the percentage of survivors its effect being most significant in the range of moderate antibiotic effectiveness With antibiotic dosage greatly in excess of minimum effective dosage depressant effect of cortisone was insignificant Cortisone interfered more with therapeutic effect of chlortetracycline (predominantly bacteriostatic and less active) than with that of streptomycin (predominantly bactericidal and more effective) (From Jawetz (71))

lence as well as the mode of action specificity toxicity and dosage of the drug

Clinical observations on the relative effects of corticosteroids and antimicrobial agents are consistent with the generalizations just expressed

A carefully controlled study of the effect of simultaneous penicillin and cortisone administration to patients with pneumococcal pneumonia showed that defervescence and symptomatic improvement occurred more promptly in patients receiving steroid and penicillin than in those given penicillin alone (148) There were no adverse bacterio

logic consequences but renal insufficiency and prolonged hypothermia developed in 1 cortisone treated patient this has been reported by others as a complication of cortisone therapy (84) The penicillin dosages used in this study were clearly sufficient to overcome any possible reduction in host resistance induced by the cortisone The results suggested that the symptomatic relief obtained with cortisone coupled with the ability of penicillin to overcome the potentially adverse effects on resistance to the pneumococci made desirable further study of combined cortisone–penicillin therapy in pneumococcal pneumonia It was recognized that similar effects might be obtained with antipyretics and analgesics such as salicylates and that further controlled study of this problem was necessary

The specific treatment of pneumococcal pneumonia poses no great problems *but when corticosteroids and antibiotics have been used for* infections in which specific treatment is more difficult, the data have not been so uniformly favorable In typhoid fever rickettsiosis and brucellosis evidence has accumulated that rapid defervescence follows the combined use of corticosteroids and specific chemotherapy with relatively little hazard to the patient However there are no carefully controlled studies such as those on pneumococcal pneumonia and it is not clear whether the improved symptomatic response does not hide an increased hazard A relation between length of cortisone treatment and the occurrence of relapses in acute brucellosis has been suggested although cortisone for 3 or 4 days was not considered to be harmful in the small series of cases studied (99)

In tuberculosis there is substantial support for combined corticosteroid-antibacterial therapy (2 22 39 72, 109 137) Patients hypersensitive to tuberculostatic drugs have been given cortisone allowing the drugs to be continued the manifestations of hypersensitivity were suppressed, without any apparent adverse effect on the course of the infection Treatment of tuberculous meningitis with corticosteroids has been attempted repeatedly on the assumption that this would decrease the incidence of late fibrotic complications and particularly of spinal block There is little doubt that corticosteroid therapy may be followed by symptomatic improvement and diminished inflammation *Unfortunately few of these observations have been controlled* and the remarkable improvements in tuberculosis management in the past few years have made it especially necessary to determine whether corticosteroids have actually contributed to the ultimate outcome in patients treated with a combination of corticosteroids and chemo

therapeutic agents In one controlled study on 30 patients neither benefit nor harm was found to result from such combined therapy (7) Further studies of this problem are in progress and additional information on the clinical usefulness of corticosteroids in tuberculosis should soon be forthcoming

Thus although there may be occasional difficulty when corticosteroids are used with effective antibiotics substantial symptomatic relief will commonly occur On the other hand many infections most notably those due to staphylococci and to certain gram negative rods have been hard to manage with the drugs available at present Conceivably corticosteroids may exert a more pronounced adverse effect in such cases simply because the antibacterial drugs are not as effective No adequate clinical data for analysis of this phase of the problem are as yet available

One study has been widely quoted as supporting the thesis that in severe infections judicious use of corticosteroids together with appropriate antibacterial agents is highly beneficial clinically (70) The series consisted of 42 patients with "medical" and 41 with "surgical" infections The medical infections included 14 cases of meningococcal infection with 4 deaths of the 4 children under the age of 18 months (the age group with the highest mortality due to this infection) 3 died Of the 14 patients 2 were considered to have benefited definitely and 3 probably from the use of corticosteroids 1 of the "benefited" group died There were 6 cases of nonmeningococcal meningitis with 2 deaths 3 were due to Hemophilus influenzae and 2 of them experienced a slow defervescence These results are not unusual and hardly suggest a particular benefit from combined corticosteroid–antibiotic therapy

In the surgical group of 41 patients the best results were obtained in the patients with ruptured appendices No infants were included in the latter group and only 2 adults 1 of whom was over the age of 65 Since such an age distribution is the optimal one for a favorable outcome in appendicitis it is hard to judge whether any benefit was obtained from the corticosteroid therapy Results in patients with perforated viscera or with breakdowns of resections with sepsis were poor In clinically difficult situations therefore there was no apparent benefit from corticosteroid therapy

The studies as summarized here emphasize the difficulties of evaluating results of treatment of infectious diseases and indicate the need for more controlled observations Despite the enthusiastic claims

made for combined corticosteroid-antibiotic therapy the evidence so far reported is not impressive These difficulties are also brought out in studies reported recently by Lepper and Spies (93) Corticosteroids were used in their hospital over a 6 year period in over 1 000 patients no marked benefit could be ascribed to their use, and some of the results might even be interpreted as showing detrimental effects Although the control methods in their study were not ideal as they realized, there were periods when none of their patients received corticosteroids for comparison Lepper and Spies are not at all enthusiastic about the value of the adrenal hormones in their patients including those with meningococcemia and tuberculous meningitis

The use of antibacterial drugs has been advocated as a routine prophylactic measure in patients receiving corticosteroids The general problem of chemoprophylaxis of infections is too complex to discuss in detail here Jordan and Dingle (74) have recently reviewed it. Suffice it to say that chemoprophylaxis has proved effective when directed toward eliminating highly susceptible organisms such as group A hemolytic streptococci or meningococci, but has not been effective in dealing with staphylococci or with some of the gram negative bacteria which readily become drug resistant. It is noteworthy that prophylactic regimens were used in many of the clinical studies in which infections were an important complication of corticosteroid therapy The infections have most commonly been staphylococcal, although coliform, monilial, and similar antibiotic-resistant infections have been encountered Prophylactic regimens can hardly be expected to confer adequate protection against all of the common antibiotic resistant organisms and the confidence physicians may feel in using such regimens to "cover" the patient whose resistance is being depressed by the use of corticosteroids is unjustified

The evidence thus far presented indicates strongly that corticotropin cortisone cortisol, and related compounds when given in sufficiently large doses depress resistance to infections and that the capacity of antimicrobial drugs to overcome this depressed resistance is limited When such limitations are not operative antimicrobial drugs frequently overcome the depressed resistance

EVIDENCE OF BENEFICIAL EFFECTS FROM CORTICOSTEROIDS

ADRENAL INSUFFICIENCY The value of adrenocortical hormones in adrenal insufficiency is so well established that detailed comment is

superfluous Recently the value of adequate replacement therapy in adrenal insufficiency has been reaffirmed (21 40 67) The results of a simple experiment from our laboratory (Fig 4) illustrate the response of the infected adrenalectomized mouse to cortisone Uninfected adrenalectomized mice of the strain used can be maintained well with 5 to 10 μg cortisone daily A dose of specific antiserum was selected which protected 50 per cent of nonadrenalectomized mice against approximately 50 000 virulent pneumococci The same dose of anti serum protected 50 per cent of adrenalectomized mice against only

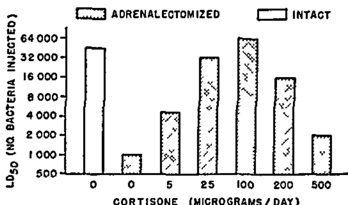


FIG 4—Effect of cortisone on pneumococcal infection in mice all given same dose of specific antiserum dose sufficient to protect 50 per cent of intact mice against 50 000 virulent pneumococci Adrenalectomy markedly enhanced suscepti bility to pneumococcal infection as shown by the much lower LD_{50} Cortisone (5 or 25 μg /day) partially restored resistance of adrenalectomized mice whereas dose of 100 μg increased LD_{50} essentially to that of control mice Larger doses of cortisone reduced resistance as shown by decrease in number of pneumo cocci necessary to produce fatal infection

about 1 000 pneumococci Administration of 5 μg cortisone per day to such mice significantly improved their resistance given about 25 μg per day their resistance returned to nearly that of the intact animals Effects of doses ranging between 25 and 100 μg cortisone per day were almost optimal but with larger doses resistance diminished and 500 μg depressed resistance to a degree comparable to that produced by adrenalectomy

Thus infection places greater demands for steroid on the host as is well known In addition the optimal dose of corticosteroid is critical and an excess is likely to be harmful Objective means for determining the optimal dose clinically are not yet available

Does adrenal insufficiency occur as a consequence of severe sepsis in the absence of pre existing Addison's disease? There are relatively few data bearing directly on this problem Traditionally autopsy evidence of tubular degenerative changes and hemorrhages in the adrenal cortex has been taken as evidence of adrenal insufficiency during life The existence of acute adrenal insufficiency has also been surmised from the clinical appearance of the patient with severe sepsis and vasomotor collapse However there is reason to suspect that adrenal insufficiency is relatively rare in severe sepsis In the first place tubular degeneration and hemorrhagic changes can be produced by intense stimulation of the adrenal gland with corticotropin (69) and not only as a consequence of exhaustion hypoxia or of direct attack by toxic agents Secondly there is evidence that vascular collapse during severe sepsis is usually not due to adrenal insufficiency (38 103) Thirdly the circulating hydroxycorticoid levels in patients in collapse due to sepsis have been measured in this laboratory and the levels have almost always been in the high normal or distinctly elevated ranges similar levels have been found in infections without collapse Levels of hydroxycorticoids comparable to those found in patients with Addison s disease have not yet been recorded in patients with severe sepsis

Direct effects of bacteria or their toxins on the adrenal cortex conceivably may lead to adrenal insufficiency However pretreatment with cortisone protects the adrenals from the damaging effects of diphtheria toxin, but does not protect the animals from the lethal action of the toxin (5 46) Presumably the damage caused by the toxin is so widespread that protection of the adrenal gland alone is of little benefit to the host

It has recently been shown that endotoxins from gram negative bacteria inhibit steroidogenesis in the perfused adrenal gland (127) The amounts of endotoxin used in the perfusion experiments are relatively large by *in vivo* standards Nevertheless the possibility of direct damage to the adrenal as a cause of adrenal insufficiency cannot be discounted despite the fact that circulating corticosteroid levels in the animals given lethal doses of endotoxin are generally above the normal range (103 127)

At present the problems posed by the foregoing discussion must be solved by direct observations of patients in the clinic Perhaps no clinical syndrome is more often considered to be associated with adrenal insufficiency than meningococcemia with collapse and the use of

cortisone and related substances is generally recommended as part of the treatment of this syndrome However, controlled observations of the value of adrenocortical hormones in this situation are difficult to find After the introduction of the first adrenocortical extracts which were extremely weak by present standards there was enthusiastic support for the use of such extracts in this condition (110) After desoxycorticosterone was introduced another cycle of enthusiasm built up only to disappear later (110) General enthusiasm for the use of corticosteroids in meningococcemia now runs high but there have been failures after the use of such steroids (93), and the collapse in this infection has been successfully treated with norepinephrine without corticosteroids (134) The largest recent series of cases of meningococcal infection to be reported is that of Tobin (146) who studied 63 cases occurring in a military experience 9 patients were considered to have Waterhouse Friderichsen syndrome and 5 survived Tobin did not find the effects of cortisone to be spectacular The experience of Jahn *et al* (70) already analyzed also does not constitute impressive evidence of the value of corticosteroids in meningococcal infections

Relative adrenal insufficiency may occur if infection appears in patients in whom exogenous steroids have suppressed steroidogenesis Increasing the dose of corticosteroid is justified under such circumstances On the other hand, when patients have received corticosteroids for a time and then begin to deteriorate the deterioration is often due to unrecognized infections Increasing the dose of corticosteroids or using corticotropin under such circumstances is likely to aggravate the infection still further unless specific antibacterial therapy is begun The amount of corticosteroid to be given to patients in whom relative adrenal insufficiency is suspected must still be determined empirically As methods for determining circulating corticosteroids become simpler it may be possible to achieve a more objective basis for determining optimal dosage level of corticosteroids Even then determination of the level of circulating steroid at any instant may not necessarily reflect the rate of production or of turnover in the host, so that most detailed study will be needed for an adequate answer to this question

In summary adrenal insufficiency is apparently a rare complication of severe sepsis There is relatively little objective support for the concept that adrenal insufficiency is common in severe infections and that therefore the use of corticosteroids is justified on that basis

EFFECTIVENESS OF SMALL DOSES OF CORTICOSTEROID —Large doses

have been used in most studies of their efficacy in infectious disease However a few observations have suggested that relatively small doses may increase resistance to infection in adrenalectomized, as well as intact, animals (124) This effect, when it has been observed has been elicited in a narrow range of steroidal dosage a slight increase above the optimal amount led to decreased resistance to infections The salutary effect of small, carefully chosen doses of cortisone has been explained on the basis that in severe infection there may be mild relative adrenal insufficiency which is overcome by giving small additional amounts of corticosteroid There is also reason to suppose that, with infection there is increased turnover of corticosteroid (122) it has long been recognized that doses of corticosteroid sufficient for maintenance of function in adrenal insufficiency may be inadequate in the presence of infection, trauma and other conditions)

On the other hand, it has already been noted that corticosteroid blood levels are usually elevated in patients during severe infections hence it must be postulated that *(1)* adrenal insufficiency is rare or *(2)* it is species specific or related to some unique feature of the experimental situation or *(3)* entry of steroid into cells is diminished, or *(4)* the increased turnover rates during infection are so great that relative insufficiency in tissues might occur in the presence of elevated blood levels Under certain of these circumstances small doses of exogenous hormone might conceivably cause more steroid to enter tissue cells The highly speculative nature of these considerations indicates how little is known about the mechanism of action of steroid hormones (60) Experimental data to support the concept that small doses of corticosteroid may be more effective than large doses in increasing resistance to infection are still inadequate and inconclusive

Can Certain Adverse Effects of Corticosteroids Be Overcome Selectively?—The augmentation of host defensive mechanisms by the use of specific antimicrobial drugs or by specific antisera when corticosteroids are being administered has already been discussed A few observations have suggested that it may be possible to separate the anaclastic effects of adrenocortical hormones on resistance to infection from some of their other metabolic effects

Thus administration of large doses of corticotropin to mice and to hamsters did not substantially depress resistance to infection when compared with the effects of cortisone or cortisol, despite the fact that the doses given caused greater loss of weight than did the cortisone (83) Smith and co-workers have observed that certain doses of corti

cotropin depressed resistance of rabbits to experimental tuberculosis less than did cortisone although corticotropin caused more marked leukopenia than cortisone (6 149) Similarly amounts of corticotropin causing significant weight changes in rabbits caused relatively little depression of antibody response whereas doses of cortisol causing less change in weight induced greater depression of antibody response (82)

There may be several explanations for these unusual findings For example the adrenal response to corticotropin varies in different animal species The adrenal glands of mice react atypically to corticotropin manifesting relatively few morphologic changes even after extensive stimulation (86) thus it is conceivable that their adrenal secretory response to corticotropin is qualitatively as well as quantitatively different from that in other animals Inferential evidence that this is true is derived from studies on the nature of adrenal secretion in different animal species it varies in different animal species and among individuals within a species (23 62 79) Although cortisol or corticosterone or both may predominate in the adrenal secretion of all animal species studied the two hormones are not necessarily equivalent in their biologic effects The relative ineffectiveness of corticosterone when compared with cortisone or cortisol in depressing resistance to infection inflammation and antibody formation or in relieving symptoms of acute arthritis is well established (26 85 114) The effects of corticotropin may differ under different circumstances in the rabbit at least, the effects of corticotropin on antibody production and on lymph nodes parallel changes in the corticosterone–cortisol ratio secreted (78) Species differences in the adrenal secretions pattern may therefore explain in part why corticotropin fails to produce changes regularly comparable to those produced by cortisol or cortisone

By the same token quantitative as well as qualitative differences in the nature of the adrenal response may influence several parameters of the metabolic response to stimulation with corticotropin The influence of differences in adrenal secretion pattern on resistance to infection cannot be precisely evaluated at present Evidence suggests that there may be individual differences in the relative amounts of corticosterone and cortisol secreted by the human adrenal cortex (125) There is also evidence that tuberculous rabbits may secrete relatively more corticosterone than cortisol, when compared with uninfected animals (79) However comparisons of the effects of exogenous

steroids on resistance mechanisms have failed to take into full account differences in absorption and metabolism of these steroids Thus corticosterone and cortisol were approximately equivalent in their protective action in rats given endotoxin when the steroids were injected intravenously but corticosterone was markedly less effective after subcutaneous injection (94) the differences after subcutaneous injection were best explained as being due to a difference in the absorption rates from the local sites of deposition Differences in the metabolic rates of injected corticosteroids have also been observed in man (112)

Other hormonal and nonhormonal substances may overcome the effects of cortisol or cortisone The adrenal secretion contains many "trace substances" the activity of which is largely unexplored and the possibility that some as yet unidentified substances may be active in overcoming certain metabolic effects of cortisol or cortisone cannot be discounted Study of one of the trace substances revealed it to be aldosterone, with electrolyte activity so great that the small amounts secreted are sufficient to account for a large share of adrenal control over mineral metabolism It is noteworthy that aldosterone exerts antagonistic effects to cortisol in sites of inflammation (31 131) and has been reported to be effective in maintaining vascular tone under experimental conditions in which cortisol desoxycorticosterone corticosterone and corticotropin have failed (101)

Somatotropic hormone has also been reported to overcome the adverse effects of cortisol or cortisone on mechanisms of resistance to infection although the evidence in support of this action is contradictory (46 54 76 77 92 93 130) Once again potency of preparation method of administration and similar artificial aspects of the experimental approach probably play a large role in determining the results However there is no doubt that potent preparations of somatotropin may overcome many metabolic effects of adrenocortical hormones (85) At present there seems to be no clear clinical role for somatotropin as a means of overcoming adverse effects of corticosteroids

An occasional report has indicated that liver extracts choline and cobalamine might overcome some actions of cortisone (65 102) but the data have been seriously challenged and the possibility that these substances might overcome adverse effects of cortisone or cortisol must be considered unproved (128 139)

Substances which may inhibit corticosteroid action would be of great interest and it is likely that inhibitors of certain metabolic ac

tions of cortisone will be found Experimental indications that lymphoid cells may contain such a substance have been advanced recently by Berglund and Fagraeus (13) who found that viable cells from the thymus or spleen overcame the inhibitory effect of cortisone on antibody production

EFFECT OF CORTICOSTEROIDS ON TOXICITY —It is well established that corticosteroids generally diminish clinical manifestations of toxicity such as malaise myalgia fever tachycardia and tachypnea this effect has constituted one of the chief bases for investigating the value of corticosteroids in the treatment of infection The data relevant to this problem may be summarized briefly as

(1) Cortisone and corticotropin markedly diminish the febrile response to several pyrogens including those derived from gram negative bacteria (51 75 119)

(2) Presumptive evidence of an effect of cortisone on hypothalamic thermoregulating centers may be derived from the finding that hypothermia occurs regularly in experimental animals and occasionally in man after administration of cortisone (33 84 148)

(3) Prolonged administration of cortisone may lead to a rise in the fever or to variable responses to pyrogens the reason for these peculiar effects is so far obscure (11 31)

(4) After pyrogens are injected into rabbits a substance which can be differentiated from the injected pyrogen appears in the blood and is pyrogenic when transferred to other animals This endogenous pyrogen appears in the cortisone treated and in the untreated animal in response to an injection of pyrogen cortisone therefore does not interfere with the formation of endogenous pyrogen (4)

(5) Larger doses of most bacterial pyrogens are lethal Cortisone cortisol and frequently corticotropin protect mice rats rabbits and chick embryos against lethal doses of endotoxins and such diverse sources as the coliform organisms Serratia marcescens various species of Brucella the typhoid paratyphoid group meningococci staphylococci and other related organisms have provided the toxic extracts (16 20 24 25 49 51 79 94 132 138 142)

(6) The protective effect of corticosteroids against bacterial endotoxins depends on increased blood levels of corticosteroids at the time of contact between endotoxin and the susceptible tissues When the steroid is given 30 minutes or more after the endotoxin the protective effect is distinctly diminished conversely if endotoxin is given after the increased blood levels due to administered corticosteroids have

returned to pretreatment values the protective effect is diminished or absent (49 94, 138)

(7) The protective effect of steroids against endotoxin is demonstrable not only with corticosterone but also with the other steroids (e g 11 hydroxyprogesterone 11-dehydrocorticosterone and even 11-desoxycorticosterone) which are generally regarded as having weak, if any anti inflammatory activity (20 25)

(8) Pretreatment with cortisone does not protect dogs and guinea pigs against endotoxin (16 37 121)

(9) Marked differences in reactivity to endotoxin and to cortisone may occur within a species In rabbits the protective effect of cortisone is demonstrable primarily in adult animals smaller rabbits (0 5 to 1 0 kg) are more resistant to the lethal effects of endotoxin, and endotoxin administration to the cortisone treated rabbit causes bilateral necrosis of the renal cortex and other evidence of a generalized *Shwartzman phenomenon In the small rabbit, therefore* cortisone supplants the usual preparatory dose of endotoxin in eliciting the Shwartzman response (143 144)

(10) Infections produced by endotoxin-containing bacteria and treated by cortisone were generally aggravated When the organisms were relatively avirulent and the dose of cortisone small, cortisone gave some protection (17) Attempts to demonstrate such protection in cortisone treated animals given toxic doses of influenza virus indicated only that cortisone aggravated the degree of injury to the host by permitting greater virus proliferation (87)

(11) Cortisone may inhibit the local response to a preparatory injection of endotoxin (142)

(12) A protective action of cortisone is not demonstrable in toxemias due to exotoxins from Clostridiae C diphtheriae C tetani C botulinum C perfringens or Shigella dysenteriae (5 17 18 47 56 108 126)

Biologically study of the effects of corticosteroids on the action of pyrogens and endotoxins is of great interest. The immediate therapeutic suggestion is that, by proper use of hormone and appropriate antibacterial agents it may be possible to interfere with the lethal action of endotoxin while controlling the infection However corticosteroids do protect all animal species against endotoxins and it is not anticipated that they would interfere with the lethal action of endotoxins already fixed to tissues The potential for harm as illustrated in the experiments on the development of bilateral renal cortical necrosis

cannot be lightly dismissed (143) There would appear to be adequate reasons therefore for considering that corticosteroids may not be helpful or may even have harmful potentials in managing infections due to endotoxin producing bacteria these must be balanced against the positive indications of the beneficial effects demonstrated both in animals and in man The need for detailed and cautious clinical study of this problem is apparent

Recently chlorpromazine and serotonin too have been found to inhibit the lethal action of endotoxins (1 55 120) The many complex interrelations involved are far from clear but there is little doubt that altered vascular reactivity, as it is affected by epinephrine and many other substances plays a large role in the physiologic changes induced by endotoxins (145) Demonstration of the effectiveness of these sub stances may suggest other avenues of approach to the management of severe sepsis

CORTICOSTEROID ENHANCEMENT OF PHARMACOLOGICALLY ACTIVE SUBSTANCES —The lack of understanding of the complex interrelations influencing vascular tone has already been stressed It is clear how ever that norepinephrine epinephrine and related agents may in crease vascular tone in states of collapse or of impending collapse although the full extent of the benefit to the patient is not yet clear Corticosteroids may potentiate the action of norepinephrine in adre nalectomized as well as intact animals thyroxine also may play a role in potentiating the action of norepinephrine and corticosteroids (91 117 151) The observation that serotonin may inhibit the lethal action of endotoxin suggests that this substance may also be involved Con versely a growing body of evidence indicates that the administration of endotoxin leads to increased sensitivity to epinephrine or serotonin so that local tissue responses to the injection of endotoxin are aug mented when epinephrine or serotonin is given (145) The apparent contradiction between the increased sensitivity to extremely small amounts of vasopressor agents in endotoxin poisoning and the use of such vasopressor agents therapeutically in large doses to restore fail ing blood pressure in patients with severe sepsis including those with bacteremia due to endotoxin producing organisms has not been re solved It is not clear whether the usually elevated levels of cortico steroid in severe sepsis are already sufficiently high to permit vaso pressor substances to exert maximal activity

The foregoing has presented both sides of the question of cortico steroid use in severe sepsis On the one hand corticosteroids depress

resistance to infection and often limit the effectiveness of antimicrobial agents in overcoming these adverse effects On the other hand, many observations indicate possible pharmacologic effects of corticosteroids which may augment resistance The need for controlled clinical observation of the net resultant effect of using steroids in infection is obvious

Although the value of corticosteroids in the management of infection is still uncertain, the attention focused on the mechanisms of host resistance and on the pathologic physiology of infections has been salutary

Corticosteroids and Inflammation and Repair

Adrenal corticosteroids are effective in diminishing the inflammatory response regardless of the stimulus Such diverse irritants as immunologically active substances (e g antigen–antibody complexes, tuberculin) trauma (burns incisions cold) chemical irritants (croton oil formaldehyde turpentine) viable infectious agents and many others have been used (50 141)

The general sequence of events in inflammation is well known injury and destruction to cells at the site of irritation, increased capillary permeability with loss of fluid and formed elements endothelial swelling and adherence of formed elements of the blood to the capillary endothelium localization of leukocytes and other cells and finally repair Each of these stages of the inflammatory response has been analyzed in an attempt to determine at which level corticosteroids influence the process (32) The existing evidence indicates that primary focus of cortisone or cortisol action is on vascular responsivity It has also been suggested that the hormones act by "preventing the release or enhancing the removal of substances coming from injured cells" and thus influence the progress of the inflammatory process (32) Administration of corticosteroids results in increased vascular tone and decreased loss of fluid and cells from capillaries adherence of leukocytes to endothelium is virtually eliminated, the subsequent collection of cells and fluid at the site of injury is diminished and as might be expected the subsequent repair is commonly less extensive and less complete (50 141)

The extent to which inflammatory changes are inhibited appears to be largely the result of an interplay between the intensity of inflammatory stimulus and the reverse action of corticosteroids although such

factors as animal species site of injection and the nature of the stimulus play a role

Duran Reynals (35) has pointed out the similarities in the response of tumors and infections to corticosteroids and has reviewed the evidence indicating that many experimental tumors are inhibited in their initial growth by hormone treatment but that the development of metastases may be promoted in the process The prolongation of survival of tissue transplants in cortisone treated animals probably reflects a similar anti inflammatory action of the corticosteroids (14)

Development of semiquantitative methods for studying inflammation has permitted more precise estimation of the relative anti inflammatory effects of various steroids hydrocortisone and related derivatives such as prednisone have been found to be superior to cortisone corticosterone and other steroids and desoxycorticosterone aldosterone and somatotropic hormone overcome the anti inflammatory action of corticosteroids to varying degrees (31 130 131) Whether the antagonism of aldosterone to the anti inflammatory corticosteroids does indeed represent an important biologic balance or is a pharmacologic action induced by excessively large exogenous doses of hormone cannot be decided at present In any event the anti inflammatory effects have been used as a basis for discovering the influence of changes in the steroidal chemical configurations on reactivity to an inflammatory irritant and several interesting and commercially useful drugs have been found in consequence (66 97)

In the course of tissue destruction and repair many chemical substances appear in the blood and indicate either developing or healing inflammation The appearance in the serum of such substances as antihyaluronidase C reactive proteins and serum mucoprotein appears to be primarily a nonspecific response to injury Administration of corticosteroids frequently results in lowered levels of many of these indices of tissue damage but it is not clear whether this decrease reflects any corticosteroid action other than a general anti inflammatory one

The effect of corticosteroids on leukocytic function is not great and generally is of insufficient magnitude to account for the adverse effects of cortisone and related substances on resistance to infection The failure of leukocytes to accumulate in a lesion is manifestly not due to peripheral neutropenia Although some diminution of phagocytic function has been noted after the use of cortisone it is not great and it has not been regularly observed Similarly cortisone causes little

if any effect on the bactericidal action of leukocytes (29 51 104 118)
The eosinopenia which generally occurs after the administration of corticosteroids is well known but the mechanism by which it occurs is obscure Lysis of the eosinophiles after exposure to cortisol has been observed *in vitro* but the effects require relatively large amounts of hormone (105 106) Lymphopenia atrophy of lymph nodes and histologic evidence of lympholysis too are regularly seen after corticosteroid administration, but the mechanism of this action is still not known and attempts to induce lympholysis *in vitro* by use of corticosteroids have not been very successful (61 63 123 129)

The effect of corticosteroids on fibrogenesis and repair has been extensively studied Clinically the hazard of delayed wound healing after corticosteroid administration appears to be relatively small, but occasionally ulcerated lesions fail to heal properly and may erode with consequent perforation or hemorrhage When large amounts of corticosteroids have been given granulations form poorly in wounds and the ingrowth of new vessels is inhibited (3 36 116) Careful observation of the latter effect has suggested that it is due to reduction of blood flow to areas of tissue damage and not to a direct cortisone effect on endothelium (3)

In general the effects of corticosteroids on inflammation and repair can be explained on the basis of their effect on vascular tone and permeability This conclusion although not yet supported by adequate evidence can provide a unifying view of the observed effects By the same token the effects of corticosteroids on hypersensitivity reactions and similar tissue responses to immunologically active stimuli probably reflect the general anti inflammatory effect of these steroids rather than a specific effect on purely immunologic mechanisms

Corticosteroids and Antibody Response (Fig 5)

There is now general agreement that cortisone and cortisol depress antibody production but do not alter the degradation rate of preformed antibody (15 44 52 82) The depressing effect of corticosterone on antibody production in rabbits is relatively slight (82)
The mechanism by which antibody production is depressed by corticosteroids is not clear Several mechanisms may be operative for example (1) the antigen may be held locally due to a vascular effect of the steroids or (2) the antibody forming mechanisms may be directly depressed or (3) the relationship of antibody forming cells to inter

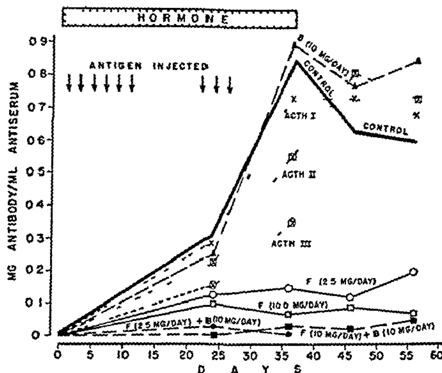


FIG 5—Effect of adrenal steroids on antibody production in rabbits Graph shows mean values for various treatment groups Cortisol (Compound F) even 2 5 mg per day markedly depressed antibody response Corticosterone (Compound B) did not overcome adverse effect of cortisol but actually seemed to have an additive effect Effect of ACTH varied with dosage 15 units daily (ACTH I) had little effect 20 mg (II) and 30 mg (III) per day respectively resulted in progressive decrease of antibody production (From Kass Kendrick and Finland (81))

mediary cells in the process of antibody production may be altered Berglund and Fagraeus (13) have presented some evidence in support of the last two possibilities The response of lymph nodes to the injection of antigen is ordinarily that of hypertrophy and hyperplasia with increased concentrations of ribonucleic acid and accumulation of plasma cells Use of cortisone inhibits the RNA response and the hypertrophy (81)

Recent detailed analyses of the effect of cortisone on antibody production have indicated that the effect of cortisone is greatest during the initial response to antigenic stimulation but that the dose of hor

mone the dose of antigen and the time of hormone administration all affect the extent of the cortisone induced inhibition (12) Of particular interest is the observation that viable spleen or thymus cells protect against cortisone inhibition of antibody production (13) Relatively intensive corticosteroid treatment is required to depress antibody production under most clinical conditions suppression of antibody production plays a relatively minor role in determining the clinical response especially in acute infections

Corticosteroids and Reticuloendothelial Function

An effect of corticosteroids on reticuloendothelial function was surmised from early studies of cortisone and corticotropin effects in ma

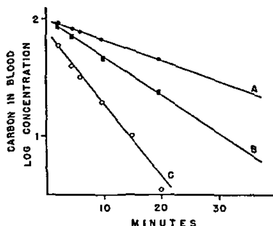


FIG 6—Effect of cortisone (12 5 mg /100 Gm body weight daily for 5 days) on return of normal clearance function in mice after saturating dose of carbon particles (16 mg /100 Gm body weight) intravenously After second dose of 8 mg carbon per 100 Gm the means of the logarithms of carbon concentrations in the blood were plotted against time *Line A* control after 5 hours *line B* cortisone treated after 3 days *line C* control after 3 days (Modified from Benacerraf *et al* (9))

laria tuberculosis and viral infections in which the activity of macrophages and of fixed reticuloendothelial cells rather than polymorphonuclear responses predominate (50) and in which corticosteroids nevertheless adversely affect resistance of the host A direct corticosteroid effect on the clearance of bacteria from the blood has not been observed (150) although when inert colloidal particles have been ad

ministered to cortisone treated animals a delay of clearance has some times occurred (28 48 64) Studies using subcutaneously injected erythrocytes (81) and tubercle bacilli inhaled by genetically defined rabbits (96) suggested that the effect of cortisone is not so much on the primary uptake of particles by reticuloendothelial cells as on the removal rate of such particles from within the cells The elegant observations of Benacerraf and co workers (9) lend substance to this view They observed that large doses of cortisone did not alter the removal rate of carbon particles from the blood stream of the mouse but that the hormone retarded the recovery rate of this function of the reticuloendothelial system as measured by the clearing of repeated doses of carbon particles (Fig 6) They suggested that the recovery of function after administration of carbon particles was due to the formation of new phagocytes and that cortisone delayed this new cell production

The important observations of Thomas and associates (142 143) on reticuloendothelial function in relation to the action of cortisone have already been cited In rabbits of appropriate size 2 successive doses of endotoxin a day apart, lead to the production of the generalized Shwartzman phenomenon That the reticuloendothelial system plays a role in this phenomenon is suggested by evidence that *(1)* tolerance to *a pyrogenic dose of endotoxin can be reversed by administration* of reticuloendothelial blocking agents and *(2)* administration of such blocking agents as killed streptococcal vaccines or thorium dioxide suspensions serves as a substitute for the preparatory dose of endotoxin in the production of the Shwartzman response Similarly cortisone may prepare the animal so that a single dose of endotoxin is sufficient to evoke this phenomenon

It may be concluded that reticuloendothelial function is disturbed by the administration of corticosteroids and that this disturbance is manifested less in the initial response of reticuloendothelial cells to an injected load than in the capacity of these cells to recover their function (or for new cells to appear) after such a load has been administered

Many attempts have been made to demonstrate *in vitro* effects of corticosteroids on isolated cells Sea urchin eggs tissue cultures of various sorts lymphocytes polymorphonuclear leukocytes macrophages erythrocytes and many other cell systems have been used (8 90 141) In general the effects observed have required the addition of amounts of steroid 10 to 100 times as great as their usual blood

levels Furthermore such *in vitro* studies have often indicated that steroids such as 11-desoxycorticosterone which is virtually inactive as an anti inflammatory agent, may be as active or more so than cortisol in producing direct effects on cells *in vitro* The assumption is that the observed effects however interesting from the point of view of cellular physiology reflect a pharmacologic action of steroids on cells quite unlike that observed in mammals given corticosteroids at physiologically active levels The failure of *in vitro* systems to reflect more precisely the observed actions of corticosteroids on various metabolic functions of the host has been a puzzling aspect of the problem and has hindered more effective studies of the mechanism of corticosteroid action

CONCLUSIONS

The value of corticosteroids in the management of infections is still not clear Only carefully controlled clinical observations are likely to solve the problem Some insight into mechanisms of host resistance and into the pathophysiology of infections have come from the studies of the effects of corticosteroids on resistance to infection The knowledge derived from these studies may eventually be more useful than the potential value of these agents in the treatment of infections At the present time it may be concluded only that the value of the adrenocortical steroids in the management of infections requires greater definition on the basis of controlled clinical study

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Primary and Secondary Hyperparathyroidism

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THE ELUCIDATION of the nature and functions of the parathyroid hormone is lagging behind that of the other endocrine secretions Clinical disorders of the parathyroid glands are not particularly common and with the diminution in resort to operation in the treatment of thyrotoxicosis and improvements in surgical technic surgical hypoparathyroidism is becoming a rarity The extract of parathyroid hormone commercially available (prepared by a method which has remained virtually unchanged for 30 years) has not proved useful in the treatment of parathyroid insufficiency and the relative efficacy of calciferol in this disorder has removed the stimulus to further research on the subject (1)

Nevertheless there are signs now of growing interest in the parathyroid glands and metabolic bone disease dating from the publication in 1948 of Albright and Reifenstein s (2) monograph on this subject and accentuated by other factors such as the appreciation of the danger of the deposition in the skeleton of fission products from nuclear explosions Above all perhaps it is the discovery confirmed by the use of labeled isotopes that the bone mineral is in a state of dynamic equilibrium with the ions of the tissue fluid which has directed attention to the metabolism of bone and emphasized our ignorance of parathyroid function

ACTIONS OF PARATHYROID HORMONE

The actions of the parathyroid hormone have been studied in the same way as those of other endocrine secretions by observing the

manifestations of spontaneous overactivity of the glands by studying the effects of injected glandular extracts, and by recording the results of parathyroid deficiency whether spontaneous or the result of surgical removal of the glands There appear to be at least two distinct actions of the parathyroid hormone—phosphaturic and calcemic

Phosphaturic Action

Assessment of Phosphaturia in Clinical Practice —The phosphate–creatinine clearance ratio (Cp Ccr) is a useful figure which can very easily be derived from the simultaneous measurement of phosphate and creatinine concentration in the serum and in the urine The ratio is derived from the equation

$$\text{Cp Ccr} = \frac{\text{urine phosphate} \times \text{serum creatinine}}{\text{serum phosphate} \times \text{urine creatinine}} \text{ (all in mg /100 ml)}$$

The resultant figure denotes the proportion of filtered phosphate which is not reabsorbed by the renal tubules

The clearance ratio does not in itself provide adequate information about phosphate excretion since it normally rises and falls with the serum phosphate (serum P) (68 69 79) This is certainly true after phosphate infusion (56) and appears to be true of changes in serum phosphate induced by variations in diet No adequate data have been reported which would show whether or not the diurnal changes in serum and urine phosphate correspond in the same way but this may be inferred from the data of Milne *et al* (69) The view to the contrary expressed by Ollayos and Winkler (77) is based on observations on only 1 subject The true relation between the serum phosphate and the clearance ratio as derived from the data of Milne *et al* is

$$\text{Cp Ccr} = 0\,64 \times \log \text{serum P} - 0\,25 \pm 0\,12$$

This equation is represented by the regression line and 95 per cent limits shown in Figure 1 in which it should be noted the serum phosphate is plotted logarithmically It is evident that the significance of the clearance ratio and of the serum phosphate in any particular case is greatly increased when the two values are considered in relation to one another Thus a clearance ratio of 0 20 falls outside the normal range if the serum phosphate is 2 0 mg per 100 ml but is normal for a serum phosphate of 5 0 A serum phosphate of 2 5 mg per 100 ml is not in itself abnormal if the urine phosphate is very low (except perhaps

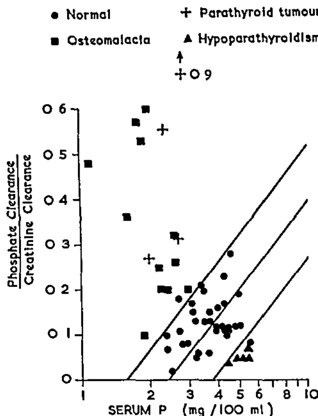


FIG 1.—The phosphate-creatinine clearance ratio and serum phosphate concentration in normal subjects and in patients with primary hyperparathyroidism, osteomalacia and hypoparathyroidism (Regression line and 95 per cent confidence limits calculated from data of Milne Stanbury and Thomson (69))

in suggesting a low phosphate intake) but it is abnormal if the clearance ratio is high

If it is assumed however that the relation of the clearance ratio to serum phosphate follows a straight line up to a serum phosphate of 8 mg per 100 ml then the following approximation holds

$$Cp\ Ccr = \frac{\text{Serum P}}{20} - 0\,07 \pm 0\,12$$

It has therefore been suggested that in any given case the observed clearance ratio should be compared with the predicted value for the

prevailing blood level derived by this formula and that the discrepancy between the two should be taken as an "index" of phosphate excretion (76) When the predicted and observed values are equal this "index" is 0 Values above +0 12 (i e, when the observed clearance ra

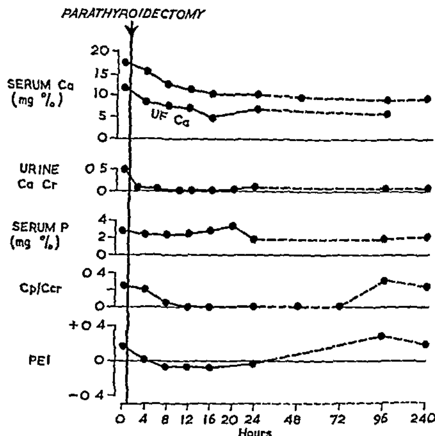


Fig 2 —Total and ultrafiltrable (*UF*) serum calcium urinary calcium creatinine ratio serum phosphate phosphate-creatinine clearance ratio and phosphate excretion index (*PEI*) before and after removal of parathyroid adenoma

tio exceeds the predicted figure by more than two standard deviations) suggest an abnormally low rate of tubular reabsorption of phosphate as in hyperparathyroidism and values below -0 12 an abnormally high rate of reabsorption as in hypoparathyroid states It is true that high figures are occasionally observed in other conditions than proved or presumed parathyroid overactivity particularly in patients with renal calculi and McGeown and Bull (59) have for this reason declared

themselves "unimpressed" by the phosphate clearance as a measure of parathyroid activity On the other hand, the present writer has not yet seen a normal value in a proved case of primary or secondary hyperparathyroidism

PHOSPHATE EXCRETION IN PARATHYROID DISORDERS The lowering of the serum phosphate with increasing degrees of parathyroid activity is associated with and probably largely or wholly due to a lowering of the renal threshold to phosphate (3 30 54 76) presumably produced by a reduction in tubular reabsorption This is borne out by the observations of Sirota (98) who showed that the phosphate–inulin clearance ratio was high in 2 cases of parathyroid adenoma and that parathyroidectomy was followed by a rise in serum phosphate and a fall in phosphate clearance Similar results have been reported by Chambers *et al* (20) by Brush (16) and by others Conversely a low clearance of phosphate by the kidneys has been observed in hypoparathyroidism (1, 68)

Figure 1 shows the phosphate–creatinine clearance ratio and the concentration of serum phosphate in 4 cases of parathyroid tumour 12 cases of osteomalacia 35 normal subjects and 5 cases of hypoparathyroidism All 4 cases of parathyroid tumour have high clearance ratio values with low concentrations of serum phosphate and in all 5 cases of hypoparathyroidism the clearance ratio is low and the serum phosphate high In 33 of the 35 normal subjects the values were within the normal limits as defined above The cases of osteomalacia discussed later are believed to be examples of secondary hyperparathyroidism The phosphate excretion index which takes both the clearance ratio and the serum phosphate into account yields the following figures for the cases represented in Figure 1

Parathyroid tumours	*Hypoparathyroidism*
+0 24	–0 12
+0 25	–0 14
+0 50	–0 15
+0 82	–0 15
	–0 13

Of the 35 normal subjects 33 fall within the normal range of –0 12 to +0 12

It should be noted that the cases of hypoparathyroidism were all receiving vitamin D and that much lower values have been observed in untreated patients

Figure 2 shows the effect of removal of a parathyroid tumour on the

serum calcium and phosphate levels and on the clearance ratio It is interesting to note that the operation did not materially alter the serum phosphate level but that the clearance ratio which was abnormally high before the operation as shown by the high "index fell rapidly to below normal levels indeed for the next 2 days the urine was virtually phosphate free The subsequent return of the clearance ratio to its preoperative level will be discussed later

The effect of clinical parathyroid disorders on the phosphate clearance or more correctly on its *normal relation to the serum phosphate* is fairly clear and is compatible with the concept that the parathyroid hormone reduces reabsorption of phosphate by the renal tubule Some confusion has been caused however because the intravenous injection of parathyroid extract does not invariably increase the urinary excretion of phosphate.

EFFECT OF INJECTING PARATHYROID EXTRACT—Albright and Ellsworth (1) *first reported the phosphate diuresis which can be provoked* by the injection of parathyroid extract and they elaborated the hypothesis that this phosphate diuresis was responsible for the calcemic action of the parathyroid hormone because it reduced the level of serum phosphate Although this hypothesis has been shown to be incorrect, the original observations on which it was based are undoubtedly valid

The effect of intravenously given parathyroid extract on urine phosphate excretion is variable

With the extract at present available some workers have definitely observed phosphaturia in normal subjects (17 75) while others have failed to produce it (60) The absolute magnitude of the response to a standard dose has been found to be fairly constant but its apparent magnitude (expressed as a percentage increase in phosphate output) *is inversely related to the rate of phosphate excretion* at the time of injection when urine phosphate was high the injection appeared to have little effect and vice versa (75) This might explain some of the negative results obtained by other workers but it is unlikely to be the complete explanation since negative results with parathyroid extract in normal subjects are reported too frequently (20 31 60) *Differences in the potency of different batches of the extract are almost certainly a factor* The effect obtained at the present time with parathyroid extract in hypoparathyroidism also appears to be less striking than that observed by Ellsworth and Howard in 1934 (34) but their cases and some of those reported by Albright and Reifenstein (2) had exception

ally high levels of serum phosphate Clearly if parathyroid extract exerts its action on the renal tubule the magnitude of such action is likely to be related to the filtered load of phosphate which is of course a function of the serum phosphate level *In this connection it is of* interest that Dent (31) found a good response to parathyroid extract only in a patient with a high serum phosphate

Administration of parathyroid extract also increases the glomerular filtration rate (18 24) and this may account for some of the phosphaturia after injection but it cannot explain the difference between the excretion of phosphate in hyper and hypoparathyroidism It has been suggested that parathyroid hormone affects the filtrability of the serum inorganic phosphate but Hopkins *et al* (44) have reported that all the serum inorganic phosphate is ultrafiltrable It is most likely that the hormone acts directly on the renal tubule presumably inhibiting phosphate reabsorption but it has also been suggested that parathyroid hormone promotes phosphate excretion by the tubule (57) Surprisingly little attention has been paid to a possible relation between the phosphaturic action of parathyroid hormone and the mechanism of acid-base regulation in the kidney particularly the excretion of titratable acid and the pH of the urine

Calcemic Action

There is no argument about the existence of the calcemic action of the parathyroid hormone The raised serum calcium of primary hyperparathyroidism is as unmistakable as that which can be induced by injections of the extract

The calcemic action of the parathyroid hormone cannot, however be explained by its phosphaturic action despite the ingenious arguments which have been put forward to support this concept the data in Figure 2 which are representative of many other observations demonstrate its fallacy In this case of primary hyperparathyroidism the serum calcium fell from a preoperative level of 17 mg per 100 ml to a normal level of 10 mg in about 16 hours without any change in the concentration of serum inorganic phosphate The low level of serum phosphate cannot therefore have been responsible for the high level of calcium It is of course true that in the typical case of parathyroid tumour the serum phosphate is low and the serum calcium high but neither serial observations in 1 case nor multiple observations in different cases yield a constant product whether calculated in terms of

secondary or tertiary calcium phosphate and there is thus no reason to believe that the changes in serum calcium are the result of changes in serum phosphate

The calcemic action must therefore be an additional effect of the hormone independent of its phosphaturic power This has been confirmed by animal experiments Stewart and Bowen (92) Ingalls *et al* (49) and others have shown that the serum calcium can be raised by parathyroid extract in dogs even after nephrectomy Barnicot (11) and Chang (21) have described the direct effect on bone of parathyroid implants and Gaillard (37) has reported an effect of parathyroid glands on bone in tissue culture In the face of this evidence some of it going back 15 years it is surprising that recent writers should still imply that the lowering of serum phosphate by parathyroid hormone is the cause of the rise in serum calcium

The intimate mechanism of the calcemic action is unknown, but it is unlikely that the hormone directly stimulates the osteoclasts to destroy bone Osteoclasis is a feature of many forms of rapid bone destruction such as Paget s disease vitamin D poisoning (4 10) and hyperthyroidism (6 36) in which there is no reason to suppose that the parathyroids are directly involved Furthermore injection of parathyroid extract raises the serum calcium before there is any histologic evidence of osteoclasis (13 14)

Howard (45) has emphasized the "equilibrium which must exist between the bone and tissue fluid minerals and has suggested that the parathyroids, by some direct action on the bone cell, regulate the level at which serum calcium is in equilibrium with bone mineral Evidence that the hormone has a direct action on the bone cell comes from Heller Steinberg (41) who observed that the first histochemical effect of injection of parathyroid extract took place around the osteocyte

It is hard to believe however that an equilibrium could exist between the bone salt and a particular ion (the calcium ion) it is more probable that some form of "solubility product" of calcium and phosphate must be operating Powdered calf bone has a reproducible "$Ca \times P$ solubility product" (74) At pH 7 4 this product is not as high as the product of calcium and phosphate in the tissue fluids of the living body but at pH 6 6 to 6 8 the product is comparable to that in mammalian tissue fluid this suggests the possibility that the pH at the bone crystal surface might be of this order In this pH range very small changes in pH cause large changes in bone "solubility" and it is conceivable that parathyroid hormone might act in this way and raise

the serum calcium by lowering bone pH Cretin (27) has in fact, suggested that the pH of bone undergoing resorption is below 7 0

There is however one obvious objection to the idea that the para thyroid hormone renders the bone salt more "soluble" it is that the product of Ca × P may be no higher in primary hyperparathyroidism when the serum calcium is high and the serum phosphate is low than in hypoparathyroidism in which the levels are reversed It must be remembered however that the bone salt probably approximates to

TABLE 1—PRODUCT OF SERUM CALCIUM AND PHOSPHATE (CA × P) IN TYPICAL HYPOTHETIC NORMAL IN HYPERPARATHYROID AND IN HYPOPARATHYROID SUBJECTS

	NORMAL	HYPERPARATHYROID	HYPOPARATHYROID
Ca	10 0 mg /100 ml.	14 mg /100 ml	5 2 mg /100 ml.
P	3 1 mg /100 ml	2.2 mg /100 ml	6 0 mg /100 ml
Ca × P	31	31	31
HPO	0 8 × 10^{-3} M /L.	0 6 × 10	1 6 × 10^{-3}
Ca^{++}	1 7 × 10^{-3} M /L	2 5 × 10^{-3}	0 9 × 10^{-3}
Ca^{++} × HPO $^{--}$	1 5 × 10^{-6} M /L	1 5 × 10^{-6}	1 5 × 10^{-6}
$(Ca^{++})^3$	5 3 × 10 M /L	15 5 × 10	0 7 × 10
PO $^{---}$	7.2 × 10 M /L.	5.5 × 10	13 6 × 10
(Ca^{++}) × (PO $^{--}$)	27.5 × 10 [illegible] M /L	39 0 × 10 [illegible]	13 5 × 10 [illegible]

tricalcium phosphate ($Ca_3(PO_4)_2$) The solubility product of such a salt is a constant related to the cube of the calcium concentration mul tiplied by the square of the phosphate, the product is therefore high er when the calcium is high and the phosphate low than when the lev els are reversed This is made clear in Table 1 which shows the product of the calcium and phosphate concentrations in a typical normal subject and in cases of hyper and hypoparathyroidism In terms of secondary calcium phosphate ($CaHPO_4$), the product is the same in all three, in terms of tertiary calcium phosphate ($Ca_3(PO_4)_2$) it is three times as high in the hyperparathyroid as in the hypoparathyroid case

The concept that parathyroid hormone exerts its effect on the pH of bone appears to conflict with the increasingly popular view that it acts by promoting the production of citrate or some other chelate in bone (63) It is possible however that acidification and chelation occur together (72)

HORMONE OR HORMONES?

Knowing that the secretions of the gland possess two distinct ac tions tempts one to speculate whether there are two parathyroid hor

mones as has been suggested by Dent (31) The first evidence that it might be possible to separate the two actions came from Davies and Gordon (29) Munson has now succeeded in separating gland extracts into fractions with different degrees of calcemic and phosphaturic action (71) So far however no correlation whatever has been established between the predominant cell type seen in parathyroid adenomas and the degree of calcemic or phosphaturic action as judged by the biochemical data of the cases concerned

STIMULUS TO PARATHYROIDS

It is clear that the parathyroid glands serve to maintain the serum calcium at about 10 mg per 100 ml in higher animals presumably to protect the *neuromuscular system against the effect of a low concentration* of calcium ions in the tissue fluid The calcium concentration in an ultrafiltrate of serum is about 6 5 mg per 100 ml (46) and it is believed that the greater part of this calcium is ionized It is reasonable to suppose that the gland is stimulated by a fall in calcium ions below a certain critical level and suppressed by a rise in calcium concentration Since the range of serum calcium is normally 9 to 11 mg % it is probable that variations above or below the corresponding range of calcium ion levels modify parathyroid activity There is indirect evidence to support this view Infusions of calcium (47 55 76 77) are followed by a fall in urine phosphate (Fig 3) and infusions of sodium citrate (76) by a rise in urine phosphate these results are believed to signify decreased and increased parathyroid activity respectively Neither of these responses is observed in the hypoparathyroid subject In terms of the "phosphate excretion index these procedures are followed by a fall and a rise respectively

It has been suggested that a rise in the serum inorganic phosphate *is also a stimulus to the parathyroids (19) but since any substantial* rise in serum phosphate depresses the serum calcium ion concentration the stimulus might be an indirect one Ham *et al* (40) have sought to solve this problem by comparing parathyroid weights in rats on different dietary regimes all deficient in vitamin D They found enlargement of the parathyroids only in those groups in which the serum calcium was reduced the high calcium and high phosphorus regime yielded high serum calcium and phosphate levels but no parathyroid hypertrophy They concluded that the stimulus to the parathyroids is a fall in tissue fluid calcium rather than a rise in phosphate and no good evidence has since been produced to prove them wrong

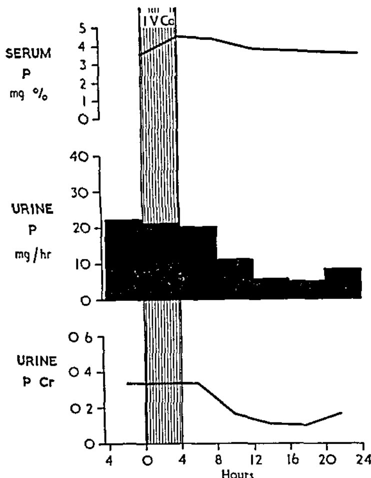


Fig 3—Effect of infusion of calcium gluconate (15 mg calcium/kg body weight) on serum phosphate and urinary phosphate and phosphate-creatinine ratio in 12 normal subjects

DIAGNOSIS OF PRIMARY HYPERPARATHYROIDISM

The clinical syndrome of primary hyperparathyroidism is now well known Presenting usually with renal calculi bone pain or nausea and vomiting, but occasionally discovered in other ways the typical patient has some or all of the following additional features polyuria weakness, hypotonia, dyspepsia and constipation The high incidence of peptic ulceration (48 82) is not always sufficiently appreciated The

serum and urine calcium levels are of course raised, unless renal failure (commonly present) is too far advanced the urine calcium being probably the more sensitive indicator of the two since a significant rise in serum calcium level presumably does not occur until the stage at which renal excretion fails to keep pace with bone destruction cases have in fact been reported in which the serum calcium was normal (64) If the extreme sensitivity of the kidney to very small changes in serum calcium level produced by calcium infusions is any guide the urine calcium may well rise before the serum concentration has increased sufficiently to be considered significant but it must also be remembered that a normal serum calcium may conceal a high ionized fraction of calcium if the amount of serum protein particularly of the albumen fraction is lowered

Refinements in diagnosis are constantly being suggested The phosphate–creatinine clearance ratio (or its converse tubular reabsorption of phosphate) has already been discussed Its use in the diagnosis of hyperparathyroidism has been suggested by Schaaf and Kyle (85) Brush (16) Chambers (20) and Crawford (24) However none of these workers takes into account the normal relation between the serum phosphate level and the clearance ratio which has been emphasized above

Howard (46 47) reported that calcium infusions caused a smaller rise in serum phosphate and a smaller fall in urine phosphate in patients with parathyroid adenoma than in normal subjects Figure 4 shows the effect of a standard calcium infusion on serum and urine phosphate in a case of parathyroid tumour the results should be compared with the normal responses shown in Figure 3 Serum phosphate rises in both cases but the fall in urine phosphate which occurs in normal subjects does not occur in the patient with primary hyperparathyroidism It is unlikely that the immediate rise in serum phosphate induced by calcium infusion is mediated by the parathyroid glands but the later fall in urine phosphate probably is (47 75) and the absence of such a fall is compatible with the presence of an autonomous parathyroid tumour which cannot be suppressed by artificially raising the serum calcium level The diagnostic value of this procedure has not yet been defined

Chambers *et al* (20) have suggested a phosphate deprivation test to confirm the diagnosis of primary hyperparathyroidism in doubtful cases they report that the rise in the tubular reabsorption of phosphate which follows deprivation in normal subjects is much less

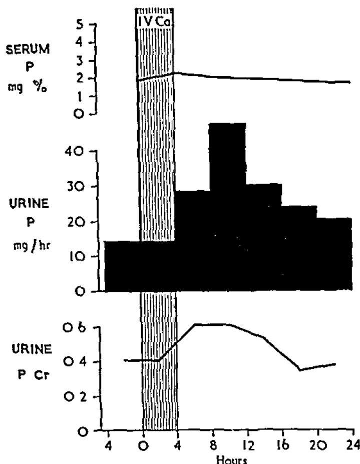


FIG 4—Primary hyperparathyroidism effect of infusion of calcium gluconate (15 mg calcium/kg body weight) on serum phosphate and urinary phosphate and phosphate creatinine ratio in patient with parathyroid adenoma

marked if a parathyroid tumour is present The suggestion is a reasonable one and deserves further testing

(4) Roentgenography may be of some assistance The lesions of osteitis fibrosa are seen only in a minority of cases the commonest bone lesion being osteoporosis (22) probably due to prolonged negative calcium balance Since the roentgenographic diagnosis of osteoporosis can be made only in the most advanced cases biopsy of bone from the iliac crest may prove useful The degree of osteoporosis present in a case

of primary hyperparathyroidism can be appreciated by comparing the photomicrograph of bone from the iliac crest with a sample of normal bone (Plate 1) It is wrong to dismiss the evidence of bone histology as negative if it does not show the features of osteitis fibrosa

Albright s suggestion (2) that the absence of the lamina dura of the teeth is of diagnostic value must be treated with reserve since a similar finding has recently been reported in Cushing s syndrome (96) Wyman and Robbins (97) have recently suggested the use of the barium swallow to demonstrate parathyroid tumours by their indentation or displacement of the oesophagus Seldinger (86) has advocated arteriography which may demonstrate a highly vascular adenoma, and Steiner *et al* (90) have applied this technic to the location of a tumour at operation The popular calcium balance test cannot be recommended as a diagnostic procedure It entails much work for all concerned without yielding more information than can be obtained from measuring the urine calcium excretion on a limited or relatively limited calcium intake

In this welter of suggested tests all of them contributing something but none of them entirely satisfactory it is well not to forget to examine the patient s neck. In the writer s experience this simple procedure has twice revealed swellings which proved to be adenomas

SYNDROMES OF SECONDARY HYPERPARATHYROIDISM

Secondary hyperparathyroidism may occur in vitamin D deficiency and in uremia

In Vitamin D Deficiency

Erdheim first reported that the parathyroid glands were enlarged in osteomalacia (35) and although some of his cases are now thought to have been examples of primary hyperparathyroidism the observation has since been confirmed by others (59 78) Parathyroid hyperplasia has also been reported in vitamin D deficient animals (43 73)

The stimulus responsible for the parathyroid overactivity comes presumably from the lowered serum calcium which occurs in rickets and osteomalacia and which is attributed by Albright and Reifenstein (2) to the malabsorption of calcium found in states of vitamin D deficiency. Low calcium diets do not however cause parathyroid hyperplasia if adequate vitamin D is given (23) and it is therefore unlikely that malabsorption of calcium alone would do so The cause of





PLATE 1 —Ph)tomicrographs of biop v sp cimens from ilnc crest *Top* from s ibject without bone di ease *Bottom* from patient with osteoporosi due to pri mary hyperparathyroidism

the parathyroid overactivity in vitamin D deficiency is probably a fall in serum calcium due to the absence of the "calcemic" effect of vitamin D on the skeleton. This very important action of vitamin D postulated by McLean in 1941 (61) has recently been demonstrated by Carlsson and Lindquist (18) in a very convincing manner They found that the administration of increasing doses of vitamin D to rachitic rats caused a progressive rise in serum calcium concentration even after no further effect was produced on calcium absorption and they conclude that vitamin D sustains serum calcium by a direct action on the skeleton.

Hypertrophy of the parathyroids is not the only evidence of parathyroid overactivity in rickets and osteomalacia Thus Lang (56) has described histologic osteitis fibrosa in osteomalacia and recently Davies and others (30) have described in osteomalacia secondary to steatorrhoea subperiosteal erosions of the phalanges indistinguishable from those seen in primary hyperparathyroidism (32) Suggestive evidence of parathyroid overactivity is provided by the raised phosphate clearance seen in osteomalacia (12 66 67 76) (*see* Fig 1) and by the fact that this high clearance can be suppressed by calcium infusion (75)

The secondary hyperparathyroidism of osteomalacia and rickets is probably an integral feature of the disease responsible for the reduced serum phosphate which is its characteristic biochemical feature. Salvesen and Boe (83) have provided some interesting evidence in this respect They found that in a large series of cases of steatorrhoea there was either a history of tetany or evidence of osteomalacia but rarely both together They suggest that tetany occurs in vitamin D deficiency only when the parathyroids fail to respond to the stimulus of a reduced serum calcium When on the other hand the parathyroids do respond they protect the patient from tetany by maintaining the serum calcium but the concomitant reduction in the serum phosphate leads to the development of osteomalacia One might add that the development of tetany in the presence of rickets suggests parathyroid "exhaustion"

It is possible that the persistent hypophosphatemia frequently seen after removal of a parathyroid tumour in the presence of bone disease should also be explained in terms of secondary hyperparathyroidism An example of this persistent hypophosphatemia is seen in Figure 2 it shows that 4 days after removal of a parathyroid tumour in a case in which there was severe osteitis fibrosa the serum phosphate was

still low and the clearance ratio high suggesting persistent parathyroid overactivity Since the serum calcium at this time was well below 10 mg per 100 ml such parathyroid overactivity was presumably secondary rather than primary A reasonable explanation of this persisting secondary hyperparathyroidism if such it is following parathyroidectomy might be that the depleted skeleton is unable to sustain the normal serum calcium concentration until some repair and *remineralisation have taken place If this is the true explanation* persistent hypophosphatemia after parathyroidectomy should be seen only in patients with bone disease as Albright and Reifenstein (2) in fact declared to be the case

In Glomerular Failure

Symmetric enlargement of the parathyroid glands in glomerular renal failure was reported by Bergstrand in 1941 (13) and is now familiar to every pathologist The fact that a form of rickets ("renal rickets) occurs in this type of renal failure is also well known (89) Albright and Reifenstein (2) showed that there were at least two forms of renal rickets" and claimed that the type associated with uremia and parathyroid hyperplasia was not rickets at all but osteitis fibrosa They postulated that the phosphate retention of glomerular failure depressed the serum calcium level and that this in turn stimulated the parathyroid glands and produced the histologic and roentgenographic features of hyperparathyroidism

It is becoming increasingly clear that this is an oversimplification It is true that osteitis fibrosa may be seen in glomerular failure but roentgenographic and histologic features of true rickets are also quite commonly seen Stanbury (89) in particular has emphasized the variety of bone histology that may be found in azotemia and has confirmed the fact that the rachitic element can be cured by large doses of vitamin D

At present it is impossible to explain the rachitic element in renal osteodystrophy and some metabolic disorder other than hyperparathyroidism must be present affecting bone It is hard to understand why osteoid should fail to calcify in uremia in the face of a normal or high Ca × P product Yendt *et al* (98) have shown that rachitic rat cartilage which will calcify in ultrafiltrates of normal serum at Ca × P products of about 30 will not calcify in ultrafiltrates of uremic serum until the product reaches about 55 and they suggest that some unspecified in

hibitor may be present in uremic serum The *only other circumstance* in which rachitic osteoid develops in the face of a high Ca × P product is hypervitaminosis D (39) but in this condition some degree of renal failure is almost invariably present and the explanation may be the same as in "renal rickets"

As for the osteitis fibrosa seen in uremia the concept that a raised level of serum phosphate depresses the serum calcium by precipitation of calcium phosphate ($CaHPO_4$) is superficially satisfactory and is compatible with the wide spread metastatic calcification seen in prolonged uremia especially in children (25 87) The solubility product $Ca^{++} \times HPO_4^{--}$ in 0 15M solutions is 3.2 when the concentration of each is expressed in millimoles per liter At pH 7 4 80 per cent of the total inorganic phosphate is present as HPO_4^{--} and about 65 per cent of the total serum calcium is probably present as Ca^{++} (depending on the concentration of albumin and globulin) The molecular weight of Ca is 40 and of P is 31 so that their concentration in milligrammes per 100 ml must be divided by 4 and 3 1 respectively when converting them into millimoles per liter the product must therefore be divided by 12 4 Thus the final calculation to arrive at the product of serum Ca and P *(mg /100 ml) which is equivalent to the solubility product* (S P) $Ca^{++} \times HPO_4$ is

$$\text{S P} \quad Ca \times P \text{ (each in mg /100 ml)} = 3\,2 \times 100/80 \times 100/65 \times 12.4 = 75$$

This figure closely corresponds to that derived in a different manner by Herbert *et al* (44) who showed that generalised metastatic calcification only occurred when the figure was exceeded

There is also evidence in the work of Meroney *et al* (65) in cases of acute anuria that the Ca × P "ceiling" in the serum is about 60 to 70 Their data (Fig 5) show the reciprocal relationship between Ca *and P in anuria and suggest a product of about this figure* over a large part of the curve It is of course extremely unlikely that this "ceiling" could be less than 60 or even as low as 60 since this figure may be reached in normal children

A Ca × P product of 75 is exceeded in the great majority of recently reported cases of metastatic calcification with parathyroid hyperplasia and osteitis fibrosa (15 25 70 80 85) and the train of events in these is comprehensible an elevated concentration of serum P causes generalized precipitation of $CaHPO_4$ with depression of the Ca concentration and stimulation of the parathyroid glands The result is mobilisation of mineral from the skeleton and its deposition in other

sites such as the arterial tree where mineral solubility is not governed by the parathyroids This is more likely to occur in children, perhaps because their Ca × P is normally higher than that of adults (Generalised metastatic calcification does not appear to occur in primary hyperparathyroidism unless the Ca concentration is excessively high (52 81) or there is associated renal failure)(7 15)

Unfortunately these considerations cannot explain the reduced

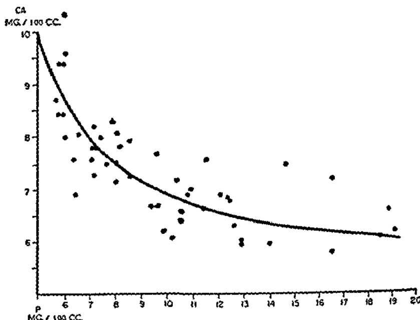


FIG 5 —Serum calcium and phosphate levels found in patients with acute anuria and regression curve calculated from observed values (From Meroney and Herndon (65))

serum Ca or even the osteitis fibrosa in all cases of uremia because it is perfectly clear that the serum Ca is often reduced below 10 mg per 100 ml before the serum P has reached the critical level or even before it is raised at all This has been the present writer s experience and that of Stanbury (89a) and confirmation of it may be found in the literature Thus Baird and Lees (8) have reported 3 cases of renal osteodystrophy in adults The first presented with osteitis fibrosa and a serum Ca reduced to 6 4 mg per 100 ml but the serum inorganic P was only 5 1 mg per 100 ml Crawford *et al* (26) reported 3 cases of osteosclerosis associated with chronic renal failure in the first and

third cases the blood urea was over 200 mg per 100 ml but the serum P was only 4.2 in one and 5 6 in the other yet the serum Ca was markedly depressed in both Similarly the first of Morgan and Mac lagan s (70) cases presented with osteitis fibrosa when the plasma P was only 5 3 mg per 100 ml It seems therefore that there is a tend ency for the serum Ca to fall in renal failure before the serum P has risen sufficiently to precipitate $CaHPO_4$ Furthermore it appears

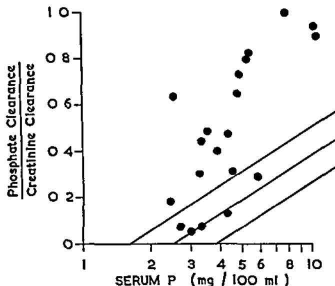


FIG 6—Serum phosphate and phosphate creatinine clearance ratios in renal failure (Calculated from data of Goldman and Bassett (38))

that the reduced serum Ca may stimulate the parathyroid glands inasmuch as osteitis fibrosa may develop in this situation

The data of Goldman and Bassett (38) also suggest the possibility that parathyroid overactivity may develop in renal failure before the serum P reaches the level of 7 5 mg per 100 ml In Figure 6 their data on phosphate excretion in renal failure have been converted into phos phate-creatinine clearance ratios and plotted in such a way that they can be compared with those in Figure 1 It is apparent that the phosphate-creatinine clearance ratio rises out of all proportion to the serum P concentration well before the concentration of the latter reaches 7 mg per 100 ml in some cases the serum P is not raised at all. This suggests that either there is some other factor present in

renal failure beside a raised serum P which may stimulate the parathyroids or that the abnormally high clearance ratio in renal failure denotes some other abnormality than parathyroid overactivity Even if the latter is true the occurrence of osteitis fibrosa before the serum P increases significantly suggests that in chronic uremia there exists some unknown metabolic factor or factors which lower the serum Ca and so stimulate the parathyroid glands Whether this is the same factor as the one responsible for the rachitic abnormality it is impossible to say but since the latter appears to respond to large doses of vitamin D (89) one is perhaps justified in speaking of a "vitamin D resistant state It is conceivable that vitamin D is inactivated or antagonised in renal failure but it is also possible that there are other anions besides phosphate which are active in causing depression of the serum Ca or that the raised citrate level in renal failure lowers the ionic calcium (78a) *A raised blood citrate could also explain the results* of Yendt *et al* (98)

PRIMARY AND SECONDARY HYPERPARATHYROIDISM IN RENAL FAILURE

It has already been stated that some degree of renal failure is very frequently present in primary hyperparathyroidism When the primary condition is left untreated the renal failure may progress until the raised serum phosphate level finally forces down the serum calcium and produces a biochemical picture indistinguishable from that of primary renal failure The presence of the bone lesions of osteitis fibrosa common to both primary and secondary hyperparathyroidism can offer no assistance in the diagnosis

This diagnostic dilemma remains unsolved but certain observations can nonetheless be made The history may be decisive If myeloma sarcoidosis the milk alkali syndrome malignant disease and vitamin D overdosage have been excluded the presence of a raised serum calcium must be taken to indicate a parathyroid tumour There is no satisfactory evidence that secondary hyperparathyroidism may go on to hypercalcemia (85) The presence of extensive metastatic calcification in the arterial tree probably favours primary *renal failure whereas* the presence of renal calcification especially in the medulla (5) probably favours primary hyperparathyroidism The urine calcium excretion cannot of course be high in renal failure but it may be high relative to the glomerular filtration rate and so equivalent to hyper

calciuria in a person with a normal kidney thus a urine calcium of over 100 mg per 24 hours in a patient with a high blood urea suggests primary rather than secondary hyperparathyroidism and it might be possible to devise a "calcium-creatinine clearance ratio" which would be of diagnostic help. It is clear that the phosphate-creatinine clearance ratio cannot be of great assistance since it is raised in renal failure but the effect of calcium infusions on phosphate excretion in renal failure might be worthy of study Another approach to the problem would be biopsy of the kidney which does not appear to have been used much for this purpose (50) However it must be admitted that the key to this diagnostic dilemma still eludes us and this is unfortunate in view of the improvement in renal function which may follow the successful removal of a parathyroid tumour (9)

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Hereditary Defects in Clotting Mechanisms*

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"Cri de coeur why does it have to be so complicated?"
—BIGGS AND MACFARLANE (39)

PERHAPS BECAUSE of some primordial human fear there has always been intense interest in the mechanisms controlling hemostasis and in abnormal bleeding states There are many early descriptions of inborn hemorrhagic disorders and crude concepts of their hereditary nature were formulated as early as the second century A D Until recently most cases of excessive bleeding were grouped under the term "hemophilia," said to have been introduced by Hopff in 1828 The intensive investigation of the physiology of clotting in the last 20 years has led to the subdivision of this syndrome into a number of different disorders This differentiation has considerable practical importance Therapy prognosis and perhaps prevention of the hereditary transmission of these diseases depend largely upon accurate diagnosis Unfortunately the physician is confronted with a voluminous literature and is confused by a complex and conflicting terminology which seems to serve as a secret code for the super specialist. The present review attempts to describe the state of knowledge concerning the hereditary hemorrhagic disorders from the point of view of the physician respon

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sible for the care of patients rather than from that of the physiologist. I have borrowed freely from the many excellent reviews which have appeared within the last few years but the material selected reflects a partisan point of view

TABLE I —Glossary of Clotting Terms

Fibrinogen Profibrin

Thrombin Thrombase Fibrin Ferment Biothrombin

Prothrombin Prothrombase Thrombogen Prothrombin B Component A

Thromboplastin Thrombokinase (some authors make a distinction between these two terms)

Pro Accelerin-Accelerin Accelerin Factor VI Activated Factor V Serum Ac celerator Globulin (Ac Globulin) Serum Accelerator Prothrombinase

The inactive precursor is called Proaccelerin Factor V Labile Factor Plasma Accelerator Globulin (Ac Globulin) Prothrombin Accelerator Plasmatic Co Factor of Thromboplastin, Prothrombin A Thrombobene Plasma Prothrombin Conversion Factor

Serum Factors Needed for the Conversion of Prothrombin to Thrombin

The terminology of these factors is currently in a state of great confusion The activated form which is responsible for the conversion of prothrombin to thrombin in association with accelerin has been called Convertin Serum Prothrombin Con version Accelerator (SPCA) Activated Thromboplastin and Stable Factor but various authors understand these terms somewhat differently The inactive pre cursors are called Proconvertin Co Thromboplastin Precursor of Serum Pro thrombin Conversion Accelerator (Pro SPCA) Prothrombin Conversion Factor Prothrombin Accelerator Co-Factor V Serum Accelerator Factors VII and X Kappa Factor Autoprothrombin I

These factors are probably multiple Two groups have been differentiated those reacting with tissue thromboplastin (Pro SPCA, Co Thromboplastin) and those which *do not appear to react with tissue thromboplastin* (Stuart Factor Prower Defect)

Antihemophilic Factor Antihemophilic Globulin Globulin Substance Plasma Thromboplastic Factor Antihemophilic Factor Platelet Co Factor I Thrombo-plastic Plasma Component Factor VIII Plasma Thromboplastic Factor A Pro thrombokinase Thromboplastinogen

Christmas Factor Plasma Thromboplastin Component Factor IX, Platelet Co Factor II Antihemophilic Factor B Plasma Thromboplastic Factor B Auto prothrombin II

Plasma Thromboplastin Antecedent Plasma Thromboplastic Factor C Factor XI

Hageman Factor Clot Promoting Factor Fifth Plasma Thromboplastin Precursor

Plasmin. Fibrinolysin Tryptase Lysin Serum Trypsin Plasma Proteolytic Enzyme

Plasminogen Proplasmin, Profibrinolysin Serum Tryptogen

Platelets Thrombocytes

PHYSIOLOGY OF BLOOD COAGULATION

A discussion of the hereditary hemorrhagic disorders requires as a frame of reference a description of the normal physiology of clotting Since knowledge of the normal state is largely derived from studies of these very disorders some circular logic is inevitable The following description is meant only as a tentative formulation of the mechanisms involved (Fig 1)

Essentially, coagulation consists in the transformation of fibrinogen,

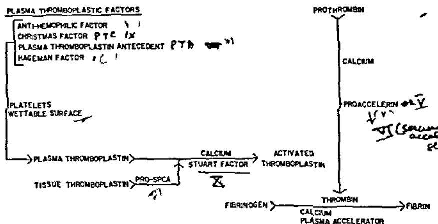
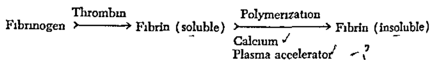


FIG 1 —Some concepts of the physiology of clotting Proaccelerin is probably converted to accelerin during coagulation. The clot accelerating effect of glass may be mediated at several stages but a major role may be its activation of Hageman factor The inhibitors of the various clotting stages have been omitted from the diagram

a soluble protein of high molecular weight, into fibrin, an insoluble network of protein fibers which traps the cellular and fluid blood elements within its meshes This transformation results from the action of thrombin a substance elaborated when blood is shed Thrombin behaves like a proteolytic enzyme (33 151 235) which alters fibrinogen by digesting the ends of its molecules The altered molecules then join together to form the interlacing fibrin strands of which the clot is composed

The action of thrombin upon fibrinogen can take place in the absence of calcium ions However addition of calcium ions in the concentration present in circulating blood greatly accelerates the coagulant effect of thrombin (216 236) and increases the clot s mechanical

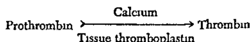
strength (80) Thus calcium ions play an important physiologic role in the final stages of clotting In addition a still unidentified substance in plasma may accelerate the reaction between fibrinogen and fibrin (211) Either this accelerator or some other substance must be present in order for calcium to exert its clot toughening effect (241) The final stages of the clotting process may be summarized as



Perhaps calcium and the plasma accelerator affect the first rather than the second of the reactions depicted

Since thrombin clots blood rapidly it cannot be present as such in the circulating blood in significant amounts Instead it is present in the form of a precursor prothrombin The synthesis of this substance apparently a glycoprotein (235) depends on the integrity of the liver (246) and requires the presence of vitamin K (190) This vitamin may function as a link in certain oxidation reduction systems (163 297) but how this results in the synthesis of prothrombin is not known

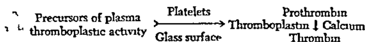
The transformation of prothrombin to thrombin when blood is shed is still not clearly understood although the outlines of the mechanisms are becoming clearer It has been known for over 100 years that particles of animal tissue accelerate clotting Schmidt postulated that these particles converted prothrombin to thrombin The active agent of tissue now called thromboplastin requires calcium to effect this transformation Teleologically speaking the mechanisms described are most useful If the body is injured the shed blood comes in contact with naked tissue which in turn initiates or accelerates the clotting process The formation of thrombin may therefore be represented as



In a simpler age this reaction was called the first stage of the clotting process although the naivete of this view was recognized at the turn of the century When blood is drawn with care to avoid the admixture of tissue juice and is then transferred to glass tubes coagulation still occurs although much more slowly than in the presence of tissue Thus blood itself appears to have the potentiality for clotting If thromboplastin is defined as any substance which initiates clotting then the blood must contain thromboplastin or its precursors The classic view

held that platelets provided this thromboplastin when blood was shed the platelets were disrupted and liberated thromboplastin, which in turn initiated clotting Since blood clots more rapidly in glass tubes than in tubes lined with paraffin, collodion or silicone the liberation of thromboplastin from platelets was thought to be accelerated by contact with a glass like surface. Actually, however platelets are only poorly thromboplastic (194 285) and their clot promoting function most probably lies in other areas.

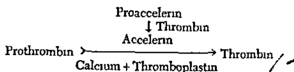
The relative simplicity of this early hypothesis was upset by evidence that the cells are not the only blood elements affected by glass surfaces If blood is drawn into syringes coated with agents such as paraffin or silicone and centrifuged in the cold, plasma essentially free of all cellular elements can be prepared without addition of an anticoagulant When the plasma is incubated in paraffin or silicone lined tubes it clots only after a long delay or not at all On the other hand, such plasma, incubated in glass tubes clots in about the same time as whole blood In other words platelet-deficient plasma contains one or more substances which, when activated by glass, initiate clotting Cell free plasma itself, then, presumably contains potentially thromboplastic activity (43 60) The platelets may enhance the activation of this thromboplastin (38) It was at first thought that there was only one soluble plasma precursor of thromboplastin, but at least four substances have now been described which may be required for the optimal development of thromboplastic activity in shed blood *(1)* antihemophilic factor *(2)* Christmas factor (or plasma thromboplastin component) *(3)* plasma thromboplastin antecedent, and *(4)* Hageman factor Hereditary disorders of blood coagulation attributable to a deficiency of each of these factors have been observed The conversion of prothrombin to thrombin must therefore be represented as



Which component is the one primarily affected by glass surfaces is disputed but evidence has accumulated that glass acts primarily on Hageman factor (212)

To complicate matters further a number of other factors have been described which seem necessary for the optimal conversion of prothrombin to thrombin Despite an overwhelming literature the nature and functions of these factors are still unclear One such substance called among many names accelerin was recognized almost simultane

ously by investigators using widely different technics (78 176 193 286) In plasma accelerin is present as a labile precursor proaccelerin which is transformed during clotting into its active form perhaps through the action of thrombin Accelerin then accelerates the con version of prothrombin to thrombin It disappears rapidly during the clotting process and cannot be found in the serum of most species This step may be outlined as



In 1904 Bordet and Gengou reported that diluted serum ac celerated the clotting of plasma Interest in this property of serum was renewed about 10 years ago (18 117) In essence normal plasma contains one or more relatively stable constituents needed to trans form prothrombin to thrombin Vitamin K is required for the synthesis of these substances and synthesis is impaired by hepatic damage or the administration of the coumarin antagonists of vitamin K Unlike proaccelerin these substances are present in serum as well as in plas ma but can be removed readily by such adsorbants as barium sulfate aluminum hydroxide and calcium phosphate

At first it was thought that a single constituent was responsible for the accelerating property of serum However evidence of the presence of more than one substance (75 247) has been confirmed very recently by studies of patients with congenital hemorrhagic disorders As a result the terminology of the stable factors is now in a state of great confusion One such factor originally called precursor of serum prothrombin conversion accelerator (pro SPCA), is necessary for op timal conversion of prothrombin to thrombin by tissue thromboplastin (18) Another, Stuart factor (113) may be necessary for the action of thromboplastin originating from tissue or from plasma Still other factors may possibly be present It is remarkable that the various serum factors as well as prothrombin and Christmas factor all have similar physical and chemical properties all require vitamin K for their synthesis and the synthesis of all is impaired by coumarin like drugs Lasch and Roka (137) and Alkjaersig and Seegers (22) have raised the pertinent question Are these substances not different stages in the evolution of a single series of proteins rather than truly separate factors? The reports that hepatic mitochondria can apparently convert proconvertin to prothrombin *in vitro* (22 137) are particularly exciting

Should this be confirmed, many of the apparent inconsistencies among experiments in this field will be resolved

Inhibitors of each of the steps of clotting have either been assumed or described Tocantins and associates (269 271–273) have studied extensively the lipoid substances in plasma which inhibit the development of thromboplastic activity in shed blood. Moreover inhibitors of thromboplastin (132 268) and of thrombin (79) have also been observed It is possible that these inhibitors help prevent the spontaneous formation of intravascular clots

Once coagulation has occurred, the fibrin network shrinks or retracts Under ordinary conditions the cellular elements of the blood remain trapped in the retracted clot, but the serum is extruded The physiologic importance of clot retraction is in doubt. Budtz Olsen (55) believes that retraction is merely an atavistic remnant from an epoch before coagulation evolved and is without significance in hemostasis However in some patients with abnormal bleeding the only demonstrable abnormality is defective clot retraction, suggesting that the phenomenon is related to hemostasis

In addition to a mechanism producing coagulation, blood contains an enzymic system which can digest clots This plasma proteolytic enzyme plasmin or fibrinolysin is present as an inactive precursor plasminogen or profibrinolysin It can be activated *in vitro* by treatment with chloroform with streptokinase (a principle found in the filtrates of certain streptococci) and with tissue extracts Moreover plasma itself may contain activators of plasminogen The enzyme can digest fibrinogen fibrin proaccelerin antihemophilic factor possibly prothrombin and certain other proteins including complement When clotted plasma is incubated under sterile conditions the clot dissolves after a variable period Rapid lysis is seen in plasma obtained from patients with a wide variety of diseases including chronic hepatic disease prostatic and pancreatic carcinoma, and shock Rapid fibrinolysis has also been noted after exercise after injection of epinephrine and in the hours immediately postpartum The role of plasmin in the bodily economy is not clear Potent inhibitors are present in the plasma There is experimental evidence that the clotting process may enhance the activation of plasmin and one wonders whether plasmin plays a part in the dissolution of small thrombi *in vivo* Rarely plasmin may become activated to such a degree during life that fibrinogenopenia and hemorrhage may result Astrup (25) has recently summarized current ideas on the physiology of plasmin

A bleeding disorder may result from a congenital or acquired deficiency of any of the elements needed for clotting It may result from the presence in blood of an anticoagulant inhibiting any of the steps in the clotting process Or it may result from the destruction of the clotting elements or of the clot itself by plasmin or a similar enzyme

SOME COMMENTS ON LABORATORY PROCEDURES
(Table 2)

Although the histories of patients with various hereditary disorders of coagulation differ in certain details ultimately diagnosis rests upon laboratory examination A review of certain tests in current use therefore seems pertinent This problem is discussed in a number of excellent texts (39 172a 262 270)

Clotting time in glass tubes—Innumerable methods have been devised to measure the lapse of time until shed blood clots These tests all attempt to determine the intrinsic capacity of blood to clot in the absence of tissue thromboplastin Many clinical laboratories still test the clotting time of capillary blood this technic will uncover only the grossest abnormalities since capillary blood is inevitably mixed with tissue juices In wider use are various modifications of the Lee and White method in which venous blood is transferred to a series of Pyrex tubes and each tube is tilted until its contents are grossly clotted The clotting time thus obtained is arbitrary serum expressed from the clot the instant that coagulation seems complete will usually re clot For reproducibility the test must be done in a standardized way using uniform clean and unetched Pyrex tubes Some lots of Pyrex tubes come from the manufacturer with a thin water repellent film which must be removed with acetone The clotting tubes must also be kept free from any silicone which may be used in the laboratory The blood must be withdrawn with great care to prevent admixture of tissue for this purpose a large bore needle is of value Other precautions include uniform filling of the tubes care not to jiggle the tubes unduly and measurement of the clotting time at a constant temperature Occasionally the clotting time will be abnormal when measured at 25 C but normal at 37 C the lower temperature is therefore preferable

There are innumerable mechanical methods many of great ingenuity for measuring clotting time At the moment the thromboelastograph devised by Hartert (102) is popular The blood to be tested is placed in

TABLE 2—DIFFERENTIATION OF SOME HEMORRHAGIC DISORDERS USUAL LABORATORY FINDINGS

DISORDERS	CLOTTING TIME	BLEEDING TIME	TOURNIQUET TEST	PROTHROMBIN TIME	SERUM PROTHROMBIC ACTIVITY	SPECIAL TESTS
Classic hemophilia	*Long*	Normal	Normal	Normal	High	Corrected by BaSO adsorbed plasma
Vascular hemophilia	Long	Long	Normal	Normal	High	Corrected by BaSO adsorbed plasma
Christmas disease	Long	Normal	Normal	Normal	High	Corrected by serum
Plasma thromboplastin antecedent (PTA) deficiency	Long	Normal	Normal	Normal	High	Corrected by serum or BaSO adsorbed plasma
Hageman trait	Long	Normal	Normal	Normal	High	Corrected by serum or $BaSO_4$ adsorbed plasma
Parahemophilia	Normal	Normal	Normal	Long	High	Corrected by fresh BaSO adsorbed plasma
Pro SPCA deficiency	Variable	Variable	Normal	Long	Normal	Corrected by serum or aged plasma
Stuart factor deficiency	Variable	Variable	Normal	Long	High	Corrected by serum or aged plasma
Hypoprothrombinemia	Normal	Normal	Normal	Long	Normal	Corrected by aged plasma
Congenital thrombocytopenia	Normal	Long	Positive	Normal	High	*Thrombocytopenia impaired clot retraction*

D tinguished by sp ial tests

a specially designed cup in which a plunger is suspended the cup is then rotated through a small arc When the clot forms it adheres to the plunger and to the wall of the cup imparting the cup s motion to the plunger The movement of the plunger is then recorded optically The utility of this clever instrument is not yet established

At best the clotting time of blood in glass tubes is a crude measure Although prolonged clotting times are found in many patients with coagulative disorders in others the clotting times may be normal despite the presence of abnormalities which are readily detectable by other means

CLOTTING TIME IN SILICONE-COATED TUBES —A major advance in the diagnosis of hemorrhagic disease was the introduction of silicone coated tubes for clotting studies by Jacques and co workers (118) Blood drawn in silicone coated syringes and transferred to silicone coated tubes takes a much longer time to clot than in uncoated tubes The same precautions needed to insure uniform results with glass tubes must be used with silicone coated tubes The clotting time is preferably measured at 25 C At 37 C the end point is often difficult to read since clot retraction may begin before clotting is complete One serious technical problem is the adequacy of the silicone coat If silicone is applied thickly the clotting time of normal blood may be so increased as to make the test impractical To obviate this difficulty the tubes may be given only a partial coating (212) This may be done with Desicote * but the coating thus obtained varies from lot to lot A satisfactory coating can be secured by rinsing tubes with 0 1 per cent Dri Film† in petroleum ether and washing away any excess with distilled water The clotting time by the siliconed tube method is one of the most sensitive indicators of clotting defects and is prolonged in virtually all cases with a detectable coagulative abnormality It might be argued that this technic should replace the glass tube method for detecting coagulative abnormalities

RECALCIFIED CLOTTING TIME —For many purposes determination of the clotting time of recalcified citrated or oxalated plasma is useful Introduced by Howell to measure prothrombin results of this test are more likely to be abnormal if there are deficiencies of factors other than prothrombin The technic employed must be uniform Its sensitivity is increased if the clotting time is measured at 25 C rather than 37 C This clotting time is often normal in patients with coagula

* Beckman Instruments Inc Mountainside N J

† General Electric Company Silicone Products Department Waterford N Y

tive disorders of considerable severity When it is prolonged the corrective effect of various normal or abnormal plasmas or serum may be tested Conversely if the clotting time is normal, the effect of small amounts of the patient s plasma upon the abnormally long recalcified clotting time of plasmas with known defects may be tested This method requires that the laboratory have available a collection of abnormal plasmas with significantly prolonged recalcified clotting times Alternately certain of the abnormal plasmas can be simulated by one or another laboratory manipulation in this case one must be constantly on guard lest unsuspected artefacts confuse the result The storage of plasmas with known defects is not without hazard Even at -70 C antihemophilic factor and proaccelerin deteriorate particularly in oxalated plasma The recalcified clotting time of oxalated plasma gradually lengthens during storage in the frozen state On the other hand for reasons that are not clear the recalcified clotting time of stored citrated plasma is often shorter than that of fresh citrated plasma *Nonetheless* the cross matching of various plasmas in the recalcified clotting time test is one of the most useful diagnostic procedures

THROMBIN TIME —The final stages of clotting the conversion of fibrinogen to fibrin may be tested by measuring the thrombin time i e the clotting time of a mixture of oxalated or citrated plasma and thrombin Any qualitative or quantitative change of fibrinogen or of the factors influencing its coagulation by thrombin may be reflected in a change in the thrombin time/ No arbitrary standard is available and a direct comparison with normal plasma must be made each time the test is performed The thrombin time of oxalated plasma lengthens after storage at -25 C and that of citrated plasma shortens The thrombin times of freshly drawn plasmas must therefore be compared or less satisfactorily of plasmas stored under reasonably similar conditions

PROTHROMBIN TIME —The middle stages of the clotting process are most readily studied by testing the one stage prothrombin time by Quick s (191) method Tissue thromboplastin is added to oxalated or citrated plasma which is then recalcified The clotting time or prothrombin time of this mixture is prolonged if there is a deficiency of any of the factors required for the formation of thrombin by thromboplastin prothrombin proaccelerin pro SPCA, or Stuart factor The prothrombin time is also prolonged in hypofibrinogenemia in afibrinogenemia no clot forms at all /

The potency of tissue thromboplastin it has been recommended,

should be such as to give a normal one stage prothrombin time of approximately 12 seconds When this is the case decreasing the concentration of prothrombin to 50 per cent of the average normal lengthens the prothrombin time by only 3 seconds Relatively greater changes will be observed with somewhat weaker suspensions of thromboplastin making it easier to recognize minor abnormalities Most laboratories compare the plasma under study with only one normal plasma but this does not take into account the considerable variation in prothrombin times among supposedly normal individuals Finally expression of the results of the prothrombin time in terms of prothrombin itself is inaccurate since the test measures a number of variables and some less committal term such as apparent prothrombic activity might be more accurate

When the one stage prothrombin time is abnormal the plasma can be analyzed to determine which factors are responsible for the abnormality The principle underlying these analyses is to mix the plasma to be tested with reagents containing all the clotting factors except the one to be measured

SERUM PROTHROMBIC ACTIVITY (PROTHROMBIN CONSUMPTION) (201 261) When venous blood is placed in glass tubes without adding anticoagulant its prothrombin is gradually converted to thrombin The rate at which this occurs depends on the rate at which thromboplastic activity develops in the shed blood and on the concentration of those factors influencing the action of the evolved thromboplastin Clotting occurs when the concentration of thrombin reaches a critical level At this time not all of the prothrombin will have been converted to thrombin Thus the prothrombic activity remaining in the blood at some arbitrary time after it is drawn may be an over all measure of the rate at which thromboplastic activity develops and the rate of the consequent conversion of prothrombin to thrombin For example in hemophilic or thrombocytopenic blood the evolution of thromboplastic activity is thought to be slower than in normal blood Reflecting this the serum prothrombic activity of such blood is higher than normal (17 201 206) This is often stated conversely namely that "prothrombin consumption is impaired"

Properly performed measurement of the serum prothrombic activity is often helpful in the diagnosis of congenital disorders of coagulation Meticulous technic is required The blood must be placed in scrupulously clean unetched Pyrex tubes free of silicone The tubes must not be agitated during incubation since this will speed the conversion of prothrombin to thrombin After an arbitrary period,

the clotting process must be stopped by adding an anticoagulant, and time then allowed for the thrombin which has evolved to be inactivated Finally the concentration of prothrombin remaining in the serum must be determined by a method which measures prothrombin specifically and is uninfluenced by other clotting factors The test should be performed in duplicate or triplicate to insure accurate results

The test for serum prothrombic activity is not sensitive and normal values may be obtained in patients with partial deficiencies of clotting factors thought to influence the determination

THROMBOPLASTIN GENERATION TEST—In 1952 Biggs and Douglas (38) introduced a test designed to measure the earliest stages of clotting, the evolution of thromboplastic activity Serum citrated plasma, and platelets are prepared from the blood of the patient to be tested and from a normal individual The plasma is adsorbed with aluminum hydroxide to remove prothrombin pro-SPCA Stuart factor and Christmas factor A mixture of platelets diluted serum diluted adsorbed plasma and calcium is prepared This "incubation mixture" containing all the recognized clotting factors except prothrombin is incubated at 37 C At intervals samples are removed and added to normal platelet deficient plasma ("substrate") This second mixture is recalcified and its clotting time determined Biggs and Douglas postulated that "thromboplastic activity" develops in the incubation mixture and that it is this activity which is measured in the second step Thus

Stage 1 Serum (containing pro SPCA Stuart factor Christmas factor and Hageman factor) + adsorbed plasma (containing proaccelerin, antihemophilic factor and Hageman factor) + platelets + calcium → "thromboplastin"

Stage 2 "Thromboplastin" + platelet deficient plasma (containing prothrombin fibrinogen and other clotting factors) + calcium → evolution of thrombin and formation of a clot.

By testing successive samples of the incubation mixture the rate of evolution of thromboplastic activity and the intensity of the activity which develops are measured

In practice the thromboplastin generation test has been most useful For example in hemophilia one can demonstrate impairment of "thromboplastin generation" which can be corrected by adding normal adsorbed plasma to an incubation mixture containing hemophilic serum and platelets However the theoretic basis of the thromboplastin generation test has been questioned The principal problem is the difficulty of preparing the ingredients of the incubation mixture free of

prothrombin There is evidence that, in addition to thromboplastin accelerin and thrombin are generated in the incubation mixture These theoretic considerations do not challenge the great utility of the thromboplastin generation test both as a diagnostic method and as an investigative tool

CLASSIC HEMOPHILIA

Classic hemophilia is a hereditary hemorrhagic disorder occurring primarily in males characterized by a tendency to bleed into soft tissues and joints upon little or no provocation It is distinguished from other hereditary bleeding diseases with similar symptoms primarily on the basis of laboratory tests These tests demonstrate that the plasma of hemophiliacs behaves as if it were deficient in a specific component, antihemophilic factor Until 1952 it was not appreciated that other hemorrhagic diseases particularly Christmas disease may mimic hemophilia For this reason it must be understood in the discussion to follow that in reports which appeared before 1953 hemophilia was lumped together with these other diseases One must be circumspect in attributing to hemophilia itself any particular quality described up to that time

INCIDENCE —The frequency with which hemophilia occurs is by no means clear Estimates vary from an incidence in Great Britain of 1 in 50 000 to 100 000 inhabitants (39) to 1 in 10 000 births in Australia (227) and 1 in 10 000 inhabitants in Holland (175a) We are personally familiar with at least 39 hemophiliacs living in Cuyahoga County the area comprising metropolitan Cleveland This is an incidence of approximately 1 in 45 000 individuals although presumably our experience does not include all the hemophiliacs in this vicinity Differences in different series may represent true geographic differences in incidence but they are certainly greatly influenced by the completeness with which an area is surveyed The difficulties in determining incidence are illustrated by conflicting statements concerning hemophilia in Negroes Albert Schweitzer (233) and others have commented upon its great rarity yet in a large series of cases Lewis and associates (150) observed no difference in the frequency of hemophilia in white and Negro families

HEREDITARY NATURE OF HEMOPHILIA (Fig 2)

In the first clear description of hemophilia Otto in 1803 recognized that the disorder was confined to males who inherited the defect from

asymptomatic females A long debate raged during the latter part of the nineteenth and early twentieth centuries concerning the heredity of hemophilia In part, the difficulty arose from the confusion of hemophilia with other bleeding syndromes The sex of an individual depends on the inheritance of a pair of sex chromosomes one from

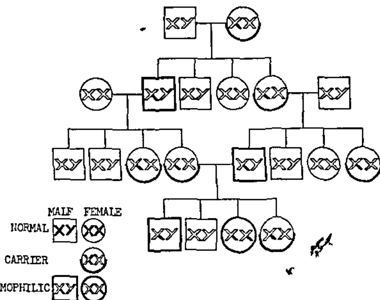


Fig 2 —The pattern of inheritance in hemophilia. The so-called sex chromosomes are represented schematically as *Xs* and *Ys* in accordance with convention. The pattern of inheritance in Christmas disease is identical

each parent The female inherits an X chromosome from each parent, so that the pair consists of two X chromosomes The male inherits an X chromosome from his mother and a much smaller Y chromosome from his father An abnormal recessive gene located on that part of the X chromosome which has no counterpart on the Y chromosome will be unopposed by a normal gene and will find expression in the developing individual In the female the same abnormal recessive gene will ordinarily be opposed by a normal gene on the corresponding portion of the second X chromosome and its effect will therefore be neutralized to a greater or lesser degree

Classic hemophilia results from the inheritance of an abnormal recessive gene located in the portion of the X chromosome unrepresented

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eration and Vogel (283) suggests a rate of 2 7 per 100,000 births How ever these figures should not be taken literally in view of the obvious difficulties in obtaining accurate human data

Lest the hereditary pattern seem cut and dried the disturbing re port of Quick and Conway (200) on a pair of identical twins similar in many measurable characteristics must be mentioned one had hemophilia the other was normal It is hard to imagine an explana tion From the data it is possible that the "hemophilic" twin actually had Christmas disease but this begs the fundamental question since the genetic problem is the same in both diseases In another pair of supposedly identical twins both boys had hemophilia (167)

Diseases closely resembling human hemophilia have been described in dogs and swine In dogs the pattern of inheritance appears to be the same as in man (81 98) while in swine the disease occurs in both sexes and is transmitted by both sexes (169) Cross matching experi ments have suggested that a similar defect is present in man dog, and swine (51)

The severity of hemophilia is at least partly determined genetically In some families the cases seem uniformly mild, in others severe (39 165 202 229) Exceptions to this generalization may occur but the individual with the supposedly severe hemophilia in a family of mild hemophiliacs may have a circulating anticoagulant (*see* p 140) or have been subjected to unusual stress such as a surgical procedure The severity of the defect can often be correlated with the concentra tion of antihemophilic factor in the plasma (52, 96 165 202) Graham Brinkhous and associates (52 96) have suggested that the different degrees of hemophilia may result from the inheritance of one of a series of allelic genes each at the same locus as the normal gene each manifested clinically by hemophilia of a given severity and each associated with a corresponding plasma concentration of antihemo philic factor

Hemophilia has been referred to as a disease of males and it has been implied that female carriers are asymptomatic From the point of view of eugenics it is of great importance to identify these female carriers) Genetically three groups of individuals may be confidently designated as carriers (1) all daughters of a hemophiliac (2) the mother of 2 or more hemophiliacs and (3) the mother of 1 hemophiliac when she has other hemophilic relatives However it is impossible to predict genetically which other female relatives of a hemophiliac are carriers

Many attempts have been made to identify the female carriers on

in the Y chromosome A male with hemophilia will have the abnormal gene in the X chromosome inherited from his mother but a normal Y chromosome inherited from his father The sons of a male hemophiliac will inherit his normal Y chromosomes if their mother is normal their X chromosomes will also be normal and they will neither have hemophilia nor transmit the trait to their offspring But all of a hemophiliac's daughters will inherit his abnormal X chromosome as well as an X chromosome from their mother If their mother is normal the hemophiliac's daughters will be heterozygous for the gene of hemophilia They will all carry the trait but will not have the overt disease Such female carriers married to normal males will transmit the hemophilic X chromosome to half their sons who will be hemophiliacs and to half their daughters who will be carriers Only if a hemophilic male marries a female carrier will a female offspring inherit the full blown disorder for she may inherit an abnormal X chromosome from each parent

This hereditary pattern has been demonstrated repeatedly by the study of large families of hemophiliacs one of the most interesting of which the descendants of Queen Victoria was again reviewed by Skold (245)

In the past patients with severe hemophilia often died before reaching maturity or without offspring One might suppose that the disorder would become progressively rarer but in fact cases continue to appear with disheartening frequency Only about 50 to 75 per cent of hemophiliacs give a family history of the disease (42) The remaining cases appear to arise *de novo* The assumption that in some of these the family history is inaccurate is a tempting one Few persons know the medical histories of their families beyond their grandparents generation Particularly in mild cases even the immediate family tree may be inaccurate However an absence of a family history is as common in severe hemophilia as in mild hemophilia Another possibility considering the small size of human families is that the genetic trait passes from female carrier to female carrier through several generations before a male with the disease appears (83 192) In several families we have studied the sex distribution among the patient's relatives was such that there was little likelihood of the appearance of another case for several generations Still another possibility is that hemophilia might arise from spontaneous mutation of a gene Various calculations of the rate at which such mutations might occur have been made Haldane (100) estimated that spontaneous mutations inducing hemophilia occur at a rate of 3 per 100 000 births per gen-

riers yet mother and daughter differed grossly in symptoms and their plasma antihemophilic activity Possibly the variable expression of the carrier state is the effect of genes which modify the activity of the gene determining the synthesis of antihemophilic factor

Nature of Defect

The essential biochemical lesion in classic hemophilia is still disputed Addis (3) was probably the first to attribute hemophilia to a defect within the plasma itself He believed that thrombin evolved more slowly than normally and thought that this resulted from a qualitative change in prothrombin Then in 1931 Govaertz and Gratia (94) observed that normal plasma filtered through a Berkefeld filter accelerated the clotting of hemophilic plasma This observation was confirmed by Patek and Stetson (179) Patek and Taylor (180) then showed that a crude globulin fraction of normal plasma corrected the defect in hemophilic blood both *in vitro* and *in vivo* a similar fraction prepared from hemophilic plasma was virtually inert They postulated that the defect in hemophilia was a deficiency of a substance found in normal globulin Somewhat similar views were expressed independently by Bendien and van Creveld (29) This concept was only slowly accepted The studies of Conley and co workers (60) did much to bolster it Normal platelet deficient plasma prepared in silicone coated tubes without the addition of anticoagulant clotted readily in glass tubes but hemophilic plasma was incoagulable under these conditions Presumably the hemophilic plasma was qualitatively different from normal plasma

Experiments with a wide variety of technics have now demonstrated that hemophilic blood behaves as if deficient in a specific clotting factor The antihemophilic factor can be found in Cohn s Fraction I of plasma in close association with fibrinogen (265) in Fractions II+III IV 1 (149) and III 2 (265) The antihemophilic activity of plasma decreases during storage at refrigerator temperatures but the rate at which this occurs varies depending on the conditions used (39 184 255) Antihemophilic activity disappears during clotting and is absent in serum (71 97 179) Antihemophilic factor is not appreciably adsorbed from oxalated or citrated plasma by such substances as calcium phosphate barium sulfate or aluminum hydroxide and can thus be distinguished from Christmas factor prothrombin pro SPCA and Stuart factor It is readily destroyed by mild heating for example at

the basis of history or laboratory study (Minor symptoms of bleeding in carrier females have been reported) Birch (42) noted that some proved or potential carriers had mild or ill defined hemorrhagic symptoms but she minimized their importance Merskey and Macfarlane (167) found that known carriers claimed to bleed excessively after dental extraction more often than did normal women but other hemorrhagic symptoms occurred no more frequently than in their control group It is difficult to assess the significance of these histories since relatives of bleeders are often prone to exaggerate bleeding episodes In our own experience there are a few families in which some of the females describe minor hemorrhagic phenomena such as epistaxes ecchymoses and menorrhagia but in most families the females are asymptomatic (161)

Many investigators have attempted to identify the carrier state by laboratory technic (161) Characteristic electrophoretic plasma protein patterns have been described in some hemophilic carriers (32) but this finding has not been confirmed (291) In most studies the methods used did not measure antihemophilic factor specifically so that an abnormality in the carriers blood may have resulted from irrelevant changes On the other hand when the concentration of antihemophilic factor itself is measured the great variation among normal plasmas must be taken into account (262) Nonetheless in several studies certain carriers have been found to have partial but significant deficiencies in antihemophilic factor (70a 96 130 131 161) In 3 families in which some carriers could be identified the hemophilia was mild (96 130 161) but this need not always be the case (70a) Curiously in a family we have studied several known carriers have had hemorrhagic symptoms but have normal concentrations of antihemophilic factor Another carrier the daughter of a hemophiliac is asymptomatic but has an abnormally low concentration of antihemophilic factor (161) Her cousin who has an even chance of being a carrier genetically speaking also has a partial deficiency of antihemophilic factor (212) perhaps in her case the carrier state has been identified In this regard the report of McGovern and Steinberg (158) is of interest They studied a young girl whose maternal grandfather had mild hemophilia She bruised readily and bled excessively after dental extractions and tonsillectomy The concentration of antihemophilic factor in her plasma was about 5 per cent of normal In contrast, her mother was asymptomatic and had a normal concentration of antihemophilic factor in her plasma Thus the patient and her mother were both presumably car

surface of the platelets a phenomenon seen with such other substances as proaccelerin and serotonin

It has been implied that hemophilic blood is deficient in antihemophilic factor Tocantins and associates (269 271–273) and Johnson (123) hold that hemophilia results from an excess of an inhibitor in plasma directed against antihemophilic factor Tocantins (271) envisions that plasma contains a complex of antihemophilic factor and a lipid inhibitor During normal clotting the lipid inhibitor dissociates from the antihemophilic factor which then reacts with a platelet factor to form plasma thromboplastin In hemophilia an excessive amount of the lipid inhibitor is conjugated with the antihemophilic factor when blood is shed the inhibitor prevents the normal formation of thromboplastin His concept evolved when he found (269) that hemophilic plasma was five to eight times as inhibitory as normal plasma against homologous brain thromboplastin suggesting that an excess of antithromboplastin was the primary cause of the delayed clotting Moreover the difference in the clotting time between normal and hemophilic plasma was minimized or abolished when each was diluted with saline (273) In this experiment the antihemophilic factor in hemophilic plasma might have been dissociated from its inhibitor by dilution Moreover a fraction was separated from hemophilic plasma which shortened the clotting time of hemophilic plasma (273)

There is no doubt that in certain of Tocantins s patients an inhibitor is present in the blood for transfusions of such blood into a normal individual prolongs the recipient s clotting time However these hemophiliacs may have the type of acquired circulating anticoagulant which has been described repeatedly in this disease (*see* p 140) At present no firm conclusion can be drawn concerning this stimulating concept However Patek and Stetson (179) observed that the transfusion of 40 ml of normal plasma shortened a hemophiliac s clotting time and Alexander and Landwehr (16) noted an effect after the administration of as little as 1 ml Crude antihemophilic factor representing as little as 1 per cent of the antihemophilic activity of a normal individual significantly shortened the clotting time of hemophilic plasma Conversely the transfusion of hemophilic blood into normal individuals did not lengthen the recipient s clotting time or induce hemorrhagic phenomena (59 263) in contrast to Tocantins s findings Finally the prolonged plasma clotting time of patients with Hageman trait was shortened by dilution with saline (213) suggesting that the effect of dilution on hemophilic plasma may not be specific All these data

50 C for 30 minutes but it will partly resist 56 C for 2 5 minutes which will precipitate fibrinogen (148) permitting the separation of these two proteins It can be separated from proaccelerin only with difficulty although the bulk of antihemophilic activity is precipitated by one third saturation with *ammonium sulfate and the bulk of pro*accelerin activity between 33 and 50 per cent saturation (176) Antihemophilic factor can be differentiated from Hageman factor in the *same manner* but we have been able to prepare antihemophilic factor devoid of Hageman factor only by using the plasma of a patient with Hageman trait as the starting material (212) Methods of preparing concentrated antihemophilic factor from bovine (34 257) sheep (35) or porcine (35) plasma have been described recently Their antigenicity for man has hampered the use of these materials in therapy The site of synthesis of antihemophilic factor remains an enigma (188)

Considerable progress has been made in elucidating the mode of action of antihemophilic factor In hemophilia the conversion of fibrinogen to fibrin by thrombin measured by the thrombin time and the middle stages of clotting measured by the one stage prothrombin time are normal However the evolution of thrombin during clotting is extremely slow (3 48) so that hemophilic serum is rich in prothrombin even in patients in whom the clotting time is normal (165) *In severe cases no measurable amount of prothrombin is* "consumed" within an hour after blood is drawn (48 194) This suggests that the defect in hemophilia is in the early stages of clotting and that antihemophilic factor *is concerned with the formation of thromboplas*tin within the plasma itself Similarly poor "thromboplastin generation" can be demonstrated in the thromboplastin generation test (38) It has been suggested that antihemophilic factor and platelets react early in clotting to produce thromboplastic activity in shed blood (49 194 206) The experiments with platelet deficient plasma referred to earlier are difficult to reconcile with this view More recent studies have indicated that antihemophilic factor may react with Christmas factor early during the course of clotting and that the product of this reaction in turn may react with platelets (30)

The early view that the essential defect in hemophilia is in the platelets is no longer tenable However Mann (159) has shown that normal platelets have weak antihemophilic activity whereas platelets obtained from the blood of hemophiliacs do not This has been confirmed in our laboratory using the thromboplastin generation test (238) Presumably, the antihemophilic factor is adsorbed onto the

injury but petechiae are unusual (195) As the child begins to crawl and walk, the bleeding episodes become more frequent, Epistaxes gastrointestinal hemorrhage and hemarthroses may occur Trivial injuries may be followed by prolonged bleeding A common site of bleeding in infancy is the frenum of the upper lip which may be cut accidentally during a fall Any form of surgery may be disastrous

Bleeding during the eruption or loss of deciduous teeth is usually no greater than in normal children but is often severe with the eruption of the permanent teeth (66) Bleeding at the gingival margins is frequent, and may be responsible for the poor dental hygiene common in hemophiliacs As a result, dental extractions are often needed, and these may be attended by protracted and even lethal hemorrhage

Hemarthroses become evident as soon as the child begins to walk, and in most patients represent the most limiting complication of the disorder They were present in 11 of 20 patients studied by Quick (195) and in 90 per cent of Davidson s series (66) The typical patient with severe hemophilia limps to the doctor on crutches The joints most frequently involved are the knees ankles and elbows (267) although bleeding into any joint may occur Initially the involved joint is stiff and painful, and rapidly becomes swollen and exquisitely tender (66) The muscles crossing the joint may be in spasm Surprisingly discoloration around the joint is uncommon (42) The blood is gradually resorbed after bleeding stops and the patient slowly regains use of the joint However once hemarthrosis has occurred the joint appears to be susceptible to further bleeding as the process repeats itself permanent and crippling deformities ensue The nature of the pathologic process has been reviewed by Key (127) and by De Palma and Cotler (67) Bleeding is apparently followed by vascular hyperplasia of the synovia and thickening of capsular and pericapsular tissues resulting in a loss of elasticity of the tissues around the joint scarring and contracture The cartilages within the joint become thinned and their superficial layers eroded Granulation tissue then invades the joint from the marrow spaces The cartilage is also invaded by a panus of hyperplastic vascular synovial and subsynovial tissue Cysts form as the result of enlargement and coalescence of Weichselbaum s lacunae (67) destruction of subchondral bone and cartilage by vascular connective tissue and, in cancellous bone breakdown of atrophic trabeculae Severe atrophy of subchondral bone may lead to fracture compression and flattening of articular surfaces In some cases excresences of newly formed cartilage and immature

make it difficult to accept the view that hemophilia ordinarily results from excess of an inhibitor in plasma

The possibility that the severe bleeding of hemophiliacs may be due in part to a defect in the tissue thromboplastic activity has been suggested However Brown (53) found that an extract of a hemophiliac s brain was as effective as normal brain thromboplastin in clotting hemophilic blood

Clinical Picture

The life history of the typical, severe hemophiliac is marked by recurrent bouts of bleeding into soft tissues muscles joints the renal pelvis and the gastrointestinal tract Many of the hemorrhagic episodes appear to arise spontaneously while others seem to be the abnormal response to the common injuries of life) An excellent review of the symptomatology of hemophilia written before this disorder was distinguished from Christmas disease is that of Davidson and co workers (66)

Except for the evidences of past hemarthroses no physical characteristics mark the hemophiliac between episodes of bleeding (42) Although the hemophiliac tends to be thin (42) no particular habitus has been ascribed to this disease However there seems to have been no systematic anthropologic study of hemophiliacs Physical examination is usually uninformative The spleen has been palpable in 5 of our patients (212) an observation contrary to the experience of others (294) and one for which we have no explanation

Hemophilia may manifest itself at birth the infant bleeding to death from the trauma of birth (167) More often the first evidence of the disease may be severe bleeding after circumcision (195) although some may be circumcised without incident Bleeding from the umbilicus common in certain other hemorrhagic disorders is unusual This is the more remarkable since Hartmann and Diamond (103) showed that the defect in hemophilic blood is demonstrable in umbilical vein blood obtained at birth an observation which has been confirmed in our laboratory (44)

The parents gradually become aware of the hemophilic infant s bleeding tendency Unexplained hematomas may appear anywhere on the infant s body and may reach enormous proportions A hematoma larger than an orange is not unusual These gradually resorb without treatment Ecchymoses may also appear after little or no obvious

tention by clotted blood is common (42) the obstruction may be seen by intravenous pyelography (262) Hematuria tends to be persistent each episode lasting many days or weeks (42) despite treatment which in other situations would seem adequate (39)

Other forms of intra abdominal bleeding are common (23) Bleeding into the psoas muscles is frequent and may simulate acute appendicitis (66) or cause compression and paralysis of the branches of the femoral plexus accompanied by severe abdominal and leg pain (262) Intra peritoneal bleeding may also simulate one or another of the acute inflammatory conditions of the abdomen (239 262) In such cases exploratory laparotomy may provide the *coup de grace*

Bleeding into the nervous system is not as rare as is usually believed (5 66 103) Hematomas in soft tissues if strategically located may cause peripheral nerve injury which may lead to permanent damage Occasionally extradural subdural, subarachnoid or focal bleeding into the central nervous system may occur (5 254) Birch (42) de scribed the case of a boy who bled into the spinal cord and was paralyzed for 9 months subsequently making a partial recovery Douglas and McAlpine (74) found neurologic lesions in 5 of 75 patients with classic hemophilia In 1 case an apparently spontaneous cerebral hemorrhage occurred in a 10 year old boy resulting in right hemi plegia In another the spinal cord was compressed by a hematoma after a fall the treatment was laminectomy In 3 instances peripheral nerves were compressed by hematomas

Despite this lugubrious description the patient with severe hemo- philia does not bleed continually instead there are repeated bouts of bleeding with intervening periods of freedom (198 254) Several sites may be involved during a relapse (66) For example in 1 of our pa tients hematuria occurred while he was under treatment for a hemato ma of the thigh The causes of this apparent deterioration in hemostatic mechanisms are a subject for speculation Stefanini and Dameshek (262) speak of a seasonal incidence of bleeding relapses occurring particularly in the spring and fall while Hartmann and Diamond (103) suggest that exacerbations follow infections Emotional stress has been said to initiate episodes of bleeding (182) but we have not found this to be the case in our patients Pavlovsky (182) suggests that the varia tions in bleeding tendency are due to changes in the blood vessels rather than in the blood itself As the patients grow older they tend to be more careful perhaps accounting for the decreasing frequency of bleeding with age (23) Time may play a more fundamental role

bone are produced at the margins of joints Repeated hemarthroses may lead to an irregular increase in the width of the epiphyses resulting in the typical knobby appearance of the joint, incongruity of the articular surfaces restricted joint motion and eventually fixed deformities However bony ankylosis is unusual Hemorrhage into the hip joint may result in destruction of the head of the femur and shortening of the involved leg (42) The changes may resemble those of coxa plana (293)

The pathogenesis of the articular lesions is not clear The tissues of patients who have bled into their joints appear laden with blood pigments Key (127) believed that the changes described might be a reaction to these substances

In some cases joint deformities may follow massive hemorrhage into the muscles or tendons resulting for example in flexion deformities of the hip following hemorrhage into the psoas muscles or the development of a "claw hand after palmar hemorrhage

The roentgenographic appearance of hemophilic joints is characteristic During the acute phase a diffuse uniform haziness may be noted sharply delineated from the surrounding tissues Later the changes may simulate osteoarthritis The bony ends of the joints may broaden and the joint spaces narrow The articular surfaces become irregular with punched out defects (127) not unlike those of gout Cysts resembling soap bubbles in appearance (262) may be seen in the subchrondral and cancellous bones and the collapse of these cysts may produce angular deformities particularly in the knee Angular deformity and shortening of the bone may also arise from premature closure of the epiphysial plate The increased density of the synovial and subcapsular tissue may result in sharp demarcation of the affected joint These tissues may become calcified

Hemorrhage into soft tissues may occlude the arterial blood supply leading to atrophy or even gangrene (262) The gangrenous part sloughs off without undue bleeding (156 267) Bleeding in the soft tissues of the floor of the mouth the neck or the mediastinum may cause death from asphyxiation unless intubation or tracheotomy is performed (39 66 254) Soft tissues distended and damaged by a collection of blood may become necrotic and develop persistent and perhaps fatal infections

In severe hemophilia hematuria is a common incident occurring in up to 90 per cent of cases (66) The bleeding may arise from any part of the urinary tract (42) Severe colic presumably from ureteral dis

died within the first year 57 per cent within the first 5 years and 95 per cent by the age of 40 a similarly gloomy prognosis is provided by Andreassen s (23) statistics for Denmark, published in 1943 Current experience however is more favorable and continued progress as our technics improve may be anticipated

The hemophiliac s life in threatened in various ways He may not survive the insults of birth (167) He may become exsanguinated from a wound although loss of blood is only rarely lethal (63 66) He may bleed into a vital area perhaps strangling from hemorrhage into the soft tissues of the neck or mediastinum Rarely bleeding into the central nervous system may be fatal (167) He may succumb to persistent infection within an area made necrotic by hematomas (66) Finally living as he does under a Damoclean sword, he may become depressed and commit suicide

Diagnosis

As measured by laboratory methods the severity of the defect roughly parallels the severity of the patient s disorder (39) The clotting time of venous blood in glass tubes may be greatly prolonged, or it may be normal or nearly normal (52, 165 196 262) Surprisingly in the experience of Biggs and Macfarlane (39) there seemed to be no middle ground Among 50 hemophiliacs about half had clotting times of 20 minutes or less and about half of 30 minutes or more but none had clotting times between 20 and 30 minutes *A normal clotting time in glass tubes therefore does not rule out the presence of hemophilia* In silicone-coated tubes the clotting time is almost always prolonged (212)

In classic hemophilia the platelet count, and thrombin and prothrombin times are normal The serum prothrombic activity is usually abnormally high (69 201 248) but a normal value does not rule out the diagnosis (165 260 262) Results of the thromboplastin generation test are abnormal but the defect is corrected by substituting normal aluminum hydroxide-adsorbed plasma for hemophilic aluminum hydroxide-adsorbed plasma in the incubation mixture The diagnosis is established by demonstrating that the patient s plasma does not correct the defect in the blood or plasma of a patient with known hemophilia and vice versa (155) Depending on circumstances one or more modalities may be tested such as the ability of the patient s plasma to correct the abnormal clotting time (165) prothrombin consumption (254 260) thromboplastin generation (38) or partial thromboplastin

however for epistaxes are frequent in childhood but become less frequent or disappear as the hemophiliac reaches the teens

Fortunately not all hemophiliacs present the grim picture described the disease being much milder in perhaps a third of the patients Brinkhous and associates (51) divide their cases into 4 groups depending on the severity of the disorder classic moderate and mild hemophilia, and subhemophilia We have not attempted such a sharp division Some patients may have no symptoms until they are 4 or 5 years old or older Bleeding may follow dental extractions tonsillectomy or other surgical procedures but may not occur during the ordinary buffeting of life There may be severe and temporarily incapacitating hematomas in soft tissues but hemarthroses are rare and permanent articular changes rarer (96)

The burdens which hemophilia imposes on the personality are borne in various ways The child s parents are continually oppressed by his disease and often guilt ridden (262) The mother may sense her husband s accusation that she has caused the disease and thus the familys troubles (92) Sometimes the mother will hide the illness from her relatives or will not admit to her husband that she has a family history of hemophilia All these forces play upon the developing child Moreover he is subjected to perpetual admonishments to be careful and may become fear ridden The frequent hospitalizations punctuated by innumerable venipunctures and intravenous injections must leave their mark As he grows older the hemophiliac feeling stigmatized (92) may withdraw into himself shunning the company of others He fears any relationship with the opposite sex and if he should marry he dreads passing the trait to his offspring The chronic shame and depression may lead to suicide the method 1 of our patients chose was to slash his wrists Other patients behave as if they were trying to deny the existence of their disease like the royal bleeder who raced automobiles Some hemophiliacs wisely choose a sedentary occupation while others tempt fate and seek jobs hazardous to the unafflicted An appreciation of the emotional problems which beset these patients is clearly helpful in the management of their lifelong illness

Prognosis —*The prognosis of hemophilia is difficult to determine* The disease was confused with other bleeding disorders until so recently that it is impossible to draw conclusions from published data Moreover mild cases of hemophilia were often overlooked Now the natural history of the disease is masked by our therapeutic efforts Birch (42) in 1937 estimated that 35 per cent of 113 hemophiliacs

long as a month before administration may contain adequate amounts of antihemophilic factor However there was considerable variability among specimens and others have demonstrated a rather rapid decline in antihemophilic factor concentration in blood bank blood (38 66) Possibly differences in the technic of obtaining blood from the donors may be responsible for the differences reported (13 184) In any case at present it seems wisest to use the freshest available blood.

Unfortunately once transfused antihemophilic factor disappears rapidly (134) so that repeated transfusions for the actively bleeding patient are necessary For this reason whole blood is only of limited value unless the hematocrit level is low Instead citrated plasma has been used effectively but it is too cumbersome to prepare before each injection. Administration of plasma separated from freshly drawn blood and kept frozen until administered (11 298) is a more practical method Finally lyophilized plasma prepared from freshly citrated plasma may be used (122) Alexander (12) and Penick and Brinkhous (184) have shown that lyophilized plasma* has reasonably high anti hemophilic potency Treatment with antihemophilic factor of animal origin is in the experimental stage (157a)

In an adult, a typical therapeutic program is the initial administra tion of 500 ml of plasma followed by 100 ml. every 4 hours until bleeding has stopped Proportionately smaller doses may be used in children Others have recommended much higher dosages for exam ple 500 ml. of plasma every 4 hours (262) In the last analysis the patient s clinical picture determines whether the amount transfused is adequate Among laboratory tests the Lee White clotting time is as helpful as any Although more subtle tests have been recommended for evaluating therapeutic results (195 254 262) they are impractical except under special conditions In the presence of a weak circulating anticoagulant, certain laboratory tests will be misleading for under such circumstances transfusions may correct the defect measured in the laboratory but be ineffective *in vivo*

After bleeding has stopped it is our practice to continue therapy with plasma at a reduced rate using 50 ml every 6 to 8 hours for sev eral days

Since the work of Patek and Taylor (180) attempts have been made to treat bleeding by the injection of concentrated antihemophilic fac tor Cohn s Fraction I (149 168) widely used at first, was not uni

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* Available from Hyland Laboratories Los Angeles Calif

time (52 133) of known hemophilic plasma When fresh hemophilic blood or frozen hemophilic plasma is not available the diagnosis can be made by preparing crude antihemophilic factor from normal plasma this fraction should correct the patient's defect while a similar fraction prepared from the patient's plasma should be inert Appropriate measures must be taken to rule out the presence of a circulating anticoagulant which might vitiate the various tests Indeed circulating anticoagulants are a frequent accompaniment of hemophilia and should be sought in every case

The bleeding time is usually normal in hemophiliacs should it be prolonged, the true diagnosis may be vascular hemophilia (*see* p 143) On the other hand the wound inflicted to determine the bleeding time often opens again when the edges of the wound are disturbed Results of the tourniquet test are usually normal although it has been reported that positive results may be obtained during bleeding episodes

It cannot be emphasized often enough that normal results of current tests as performed in most laboratories do not rule out hemophilia We have repeatedly had to treat mild hemophiliacs who have bled severely after dental extraction These patients have often told us that their story was disbelieved because routine tests showed no abnormalities

Treatment

The treatment of hemophilic bleeding by the transfusion of blood or blood products was introduced early in this century by Weil (288) although Lane had attempted such measures almost a century before (132a) Weil's use of serum would now be considered inappropriate for cases of classic hemophilia With the development of technics for transfusion the treatment of bleeding by intravenous administration of normal blood plasma or purified antihemophilic factor became feasible Although practice varies from clinic to clinic the following generalizations seem valid Adequate hemostasis can often be achieved by raising the blood concentration of antihemophilic factor to 5 or 10 per cent of normal (12 13 134) Under some circumstances however levels as high as 30 to 50 per cent of normal may be needed (4 157) These levels are difficult to achieve by transfusion of whole blood if the patient has not lost an appreciable amount of blood How fresh the transfused blood must be is a debated question It would be of obvious advantage if stored bank blood were adequate Spaet and Garner (255) and Penick and Brinkhous (184) have reported that blood drawn as

relief of pain reduction of distention and a rapid return of function (153) However this form of treatment has not found general favor

As soon as it becomes apparent that bleeding has stopped motion is encouraged within the limits set by pain (67) Diathermy passive exercise and later active exercise are helpful (195) In the case of the knee traction to the lower leg is used after aspiration to minimize contraction deformities (67) The patient is then encouraged to walk on crutches for several weeks until his muscles have become strong enough to stabilize the joint If complete correction of the deformity at the knee cannot be achieved, a long leg brace with a circular spring at the knee joint is used intermittently More persistent deformities are treated by continuous skin traction or wedging plaster casts but the genu valgum and external rotation of the tibia on the femur which follow repeated hemarthroses resist treatment (67) Finally corrective operations may be performed in those patients whose bleeding tendency can be readily overcome by blood transfusion De Palma and Cotler (67) have performed arthrodesis of the knee and hip joints as well as other orthopedic surgical procedures in hemophiliacs Dr P Curtiss at the University Hospitals of Cleveland has lengthened the scarred and shortened Achilles tendon of a patient with severe hemophilia after temporarily correcting the hemostatic defect by transfusion of plasma It cannot be emphasized too strongly that such surgical procedures should be performed only in those patients who respond to administration of normal plasma and under the close supervision of someone familiar with the treatment of hemorrhagic disorders

Occasionally other surgical procedures must be performed in patients with hemophilia The serious nature of such operations has been emphasized in a comprehensive review (63) Appendectomy nephrectomy gastrectomy gastroenterostomy laminectomy repair of gunshot wounds amputation of gangrenous limbs or digits and skin grafting have all been performed successfully Nevertheless surgery should be avoided whenever possible for the over all fatality rate is high (63) and in those who have survived it is often necessary to give repeated transfusions with all the risks attending this procedure The suggestion of Davidson and associates (66) that acute appendicitis be treated with antibiotics has merit particularly since the differentiation of this disorder from intra abdominal bleeding is difficult Paradoxically patients with milder hemophilia may be in greater danger from surgery than those with severe disease for the physician may be lulled into a false sense of security by the negligible clotting abnor

formly effective because of its variable potency (250) Its use has fallen into disrepute because evidence exists that it stimulates the development of circulating anticoagulants directed against antihemophilic factor Concentrated antihemophilic factor of animal origin (34 35) may be effective but its use is attended by thrombocytopenia (157) and by evidence of the development of antibodies against the heterologous proteins a dangerous anaphylactoid reaction has been reported (84) A wide variety of drugs and hormones have been proposed for systemic therapy in hemophilia but none has been of any value

In addition to transfusion treatment must include immobilization of the bleeding area which usually means putting and keeping the patient at rest Ice bags may relieve the pain attending distention of tissues with blood and perhaps decrease the rate of bleeding by inducing local vasoconstriction (195) Ordinarily localized hematomas should not be treated surgically but one may be forced to aspirate blood from the distended tissues to prevent necrosis Strict precautions must then be taken to prevent infection De Palma and Cotler (67) believe that the injection of hyaluronidase into areas of massive hemorrhage in soft tissues will result in more rapid absorption of blood and thus prevent local necrosis

Local bleeding may be treated by application of bovine thrombin to the area (2) Biggs and Macfarlane (39) recommend the use of Russell s viper venom They caution against prolonged application of pressure to injured tissues since this may delay healing They point out that local use of coagulant solutions is effective only if hemorrhage can be temporarily arrested so that the clot may form at the surface which is actually bleeding Stefanini and Dameshek (262) suggest the use of the cautery in controlling bleeding from the nasal and buccal mucosae but we have been impressed by the frequent recurrence of epistaxes following cauterization The use of a pledget of absorbable material soaked in thrombin and pressed firmly against the bleeding point (156) may be more effective

Opinion on the optimal management of hemarthrosis differs The joint should be immobilized a compression bandage applied and the part surrounded by ice packs (67 195) If bleeding appears to continue or if the hemarthrosis is massive transfusion should be given and intra articular tension relieved by aspiration of the joint (67 298) although Stefanini and Dameshek frown upon this procedure (262) The introduction of hyaluronidase into the affected joint has been suggested to promote the reabsorption of blood the results claimed are the

margin (66) After several weeks the teeth are readily extracted However this method is said to be dangerous and is not recommended (24 76 183)

Prophylaxis in hemophilia is difficult, since bleeding may occur without obvious provocation The physician must steer between restricting the child so that his personality may be warped and allowing a freedom which results in crippling injuries Before the child can care for himself his environment must protect him The crib should be lined with soft material such as mattress padding (197) and the crib or bed should be surrounded by deep carpeting to soften the blow when he falls or jumps out Toys should be selected with an eye to their possible dangers (197) Such accident producers as the pedals of tricycles should be padded A football helmet offers some protection against hematomas of the scalp a common lesion Padded clothing may be helpful As the child grows older he should avoid body-contact and other dangerous sports Swimming (196) sailing (262) and golf may usually be pursued without difficulty Artful persuasion is necessary since the adolescent will try to imitate his peers The patient and his parents should be advised that he should be trained for a sedentary occupation it is helpful to point out that many hemophiliacs have become highly valuable citizens for their number includes musicians doctors engineers teachers and other professional people Many cities have chapters of the Hemophilia Foundation * This groups chief activities include the dissemination of knowledge about bleeding disorders to patients and physicians and provision of blood and frozen plasma for patients

Johnson (122), Alexander and Landwehr (16) and van Creveld and Paulssen (275) have suggested that repeated transfusions of small amounts of plasma may have prophylactic value in severely affected patients Alexander and Landwehr (16) inject 100 to 180 ml of plasma three times a week or oftener They believe this procedure decreases the frequency of bleeding and permits rehabilitation of the patient (16) However the frequent venipunctures required may scar the veins so that transfusions needed to control a bleeding episode may be extremely difficult to give Furthermore blood transfusion is always attended by the risk of acquiring homologous serum jaundice a complication which has been reported (16) Except under unusual circumstances therefore such prophylactic treatment is best avoided

* National office 75 Fifth Ave New York 10 N Y

malities revealed by routine testing The patient s story of a bleeding tendency should be given credence even in the face of supposedly objective data If surgery is necessary transfusions of large amounts of plasma should be administered just before during and after operation

The extraction of carious teeth is the commonest surgical procedure to which hemophiliacs are subjected *Opinion varies concerning the* best approach Biggs and Macfarlane (39) recommend that the patient be admitted to the hospital and that no more than 2 teeth be removed at a time Preferably extraction is performed under general anesthesia, and a light plug of cotton or fibrin foam soaked in Russells viper venom or thrombin is laid over the socket and held in place by the dentist s finger for about 5 minutes A previously prepared dental splint is then applied to keeping the dressing in place replacing it as needed Tight plugging of the socket is avoided so as to prevent necrosis of the edges of the wound and the gum margin is not sutured lest hematomas form which may spread and cause asphyxia Biggs and Macfarlane used transfusions only to compensate for loss of blood Others believe that the patient should be prepared by the transfusion of blood or plasma (141 262 298) After the tooth is extracted and the splint is applied for which Levine (141) uses a rapidly hardening plastic "stent" he immobilizes the jaw by placing an operating room cap on the patient s head and pinning a piece of elastic gauze to the cap to produce a slight posterior pull on the jaw The cap is not removed for 48 hours and then only during the day for a week Transfusions of blood or plasma are continued until all bleeding has ceased

Lyon (152) points out that the purpose of the dental splint is to protect the tissues in the area from disturbance by the tongue's motion by oral fluids and food and to hold medication in position He emphasizes that the splint is not intended to provide pressure upon the area which might actually increase bruising He uses topical hemostatic agents freely packing the dental socket with a piece of *Gelfoam* soaked with Thrombol subcutaneous* and powdered with topical bovine Thrombin † To control undue oozing in the days after extraction Lyon applies ferric subsulfate solution (Monsel s solution) locally as often as needed

Some have recommended that the teeth be loosened before extraction by placing thin rubber bands around their bases at the gingival

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* Merck Sharp & Dohme

† The Upjohn Company

been treated with Cohn s Fraction I, and they wondered whether these preparations were responsible for the development of anticoagulants Lawrence and Craddock (62, 138a) and Munro and Munro (171) found that the anticoagulant was associated with the γ globulin fraction, al though similar anticoagulants have been localized to the β globulin fraction as well (112) Lawrence and Craddock and many others have reported that the anticoagulant plasma contained precipitins against antihemophilic factor and therefore have proposed that the circu lating anticoagulants were antibodies and that the inhibition of clot ting resulted from an antigen-antibody reaction Lewis and co-work ers (146) have shown that the anticoagulant specifically destroys anti hemophilic factor an observation which we have corroborated (160) but which is not accepted by all (276) Experimentally human anti hemophilic factor is antigenic for rabbits and the rabbit antiserum appears to inactivate human antihemophilic factor and inhibit the formation of thromboplastin during clotting (217)

Nevertheless certain observations raise the question whether circu lating anticoagulants in hemophilia are actually antibodies A number of cases have been described in which precipitins could not be dem onstrated (61 146 243 276) and re-examination of others has failed to confirm the presence of precipitins which had been observed earlier (12) Lawrence and Craddock (138a) noted that the inactivation of antihemophilic factor by the anticoagulant serum required a period of incubation This behavior is as compatible with that of an enzyme as with that of an antibody Some insight into the nature of this problem comes from studies by Biggs and Macfarlane (39) of circulating anti coagulant against antihemophilic factor found in the blood of a woman with a postpartum hemorrhagic diathesis They found that the destruc tion of antihemophilic factor required a considerable time We have made the same observation in 2 patients with similar anticoagulants in whom the destruction of antihemophilic factor occurred with in creasing rapidity as the temperature of the reaction mixture was in creased from 0 to 37 C (161) In addition, complement fixation could not be demonstrated during the inactivation of partially purified antihemophilic factor by concentrates of the anticoagulants * Nor could we show that the anticoagulants agglutinated tanned erythro-cytes coated with antihemophilic factor Finally we observed that

* These experiments were carried out in association with Drs I Lepow and A. Stavitsky

The hemophiliac risks injury under circumstances in which he is unable to give his history to the attending physicians For this reason, patients with this disorder should carry a note giving the diagnosis We suggest to hemophiliacs that when they travel they carry a supply of lyophilized plasma The chief virtue of this is psychologic it reassures the patient or his family that emergency therapy can be started wherever blood or plasma is not readily available

Finally the patient or a potential carrier will seek advice concerning marriage and having children I believe with Davidson and associates (66) that the patient should be taught the statistical risks in having children but that the final decision is one to be made by the patient or carrier The hemophiliac s daughters will all be carriers but his grandchildren will not be born for another generation at which time our therapeutic measures may be more successful

Circulating Anticoagulants

The complication probably most to be feared in hemophilia is the development of a circulating anticoagulant that is a substance in plasma which neutralizes the effect of transfusions of normal blood Verstraete and Vandenbroucke (282) have reviewed the literature concerning circulating anticoagulants The earliest description of a circulating anticoagulant is probably that of Pickering and Gladstone (185) who observed that the blood of a patient with severe hemophilia retarded the clotting of an equal volume of normal blood In 1941 Lawrence and Johnson (139) described a 44 year old hemophiliac who was refractory to blood transfusion His blood contained a substance prolonging the clotting of normal blood Since then many similar cases have been reported indeed the presence of a circulating anticoagulant in hemophilic blood is rather common For example Frommeyer and co workers (89) observed anticoagulants in 5 of 22 hemophiliacs tested Lewis and associates (146) in 5 of 52 and Margolius and I (160 161) in 6 of 28

The etiology and nature of the circulating anticoagulants in hemophilic blood are not clear The anticoagulants cannot be extracted with ether (139 170) and thus seem not to be identical with that which Tocantins and associates (269 272) postulate to be present in all hemophilic blood To my knowledge no case has yet been described in a patient who has not previously been transfused with normal blood or a blood fraction Frommeyer *et al* (89) noted that their 5 patients had all

hemophilia occurs the mating of a hemophilic male and a carrier female does result in the birth of hemophilic females (50)

At least 4 well-authenticated cases of hemophilia in women have been reported 3 of these were in sisters the offspring of a consanguineous marriage in a hemophilic family (166) 2 other sisters were affected and 4 were unaffected but these 6 individuals were not tested in the laboratory The hemophilic women bled excessively from wounds and after dental extractions bruised easily bled from the urinary and gastrointestinal tracts and had menorrhagia yet they survived childbirth Their sons were of course hemophiliacs In this family the Lee White clotting time was normal but the patients plasmas failed to correct the defect in known hemophilic blood These patients have been shown to have classic hemophilia and not Christmas disease (166a) The parents of the fourth patient, described by Israels and associates (115) were unrelated but her father and her mother s brother were known hemophiliacs The patient s clotting time was prolonged and her plasma did not correct the abnormality of hemophilic blood However she had only minor evidences of a bleeding tendency and menstruation was not abnormal 10 days after the delivery of a normal infant at term an event unaccompanied by excessive bleeding intractable uterine hemorrhage developed for which hysterectomy was performed Despite a stormy course the patient recovered A fifth case was that of a girl whose parents were first cousins her father being a hemophiliac and her mother the daughter of another (48) the patient bruised easily and had hemarthroses but her menses were normal no laboratory tests were performed

The case of the young girl reported by Quick and Hussey (203) as an instances of hemophilia in the female should be mentioned Dr Quick (199) is no longer willing to commit himself concerning the diagnosis although laboratory tests continue to demonstrate abnormalities in the clotting mechanism

VASCULAR HEMOPHILIA

A variety of hemophilia has been described in the last few years manifested by bleeding phenomena a deficiency of plasma antihemophilic activity and evidences of a vascular disorder, principally prolongation of the bleeding time Unlike classic hemophilia both sexes are affected For example among 20 cases reviewed by Singer

the circulating anticoagulants were not specifically directed against human antihemophilic factor, as had been reported (256) but destroyed antihemophilic factor of animal origin as well None of these observations rule out the possibility that these anticoagulants or the indistinguishable anticoagulants found in hemophilic blood are antibodies, but they do suggest that studies of their mode of action must be continued

The presence of a circulating anticoagulant should be suspected in any hemophiliac who responds poorly to blood transfusion In such patients bleeding may be more protracted and severe than in the usual hemophiliac and the clotting time is often extraordinarily long (146 160 256) Various technics have been described for the detection of circulating anticoagulants The most sensitive methods seem to be those in which the patient s plasma is incubated with normal plasma for several hours without the addition of calcium and then tested (38 146 161) This technic will demonstrate anticoagulants which may not be detected without the preliminary incubation After an anticoagulant has appeared it is not invariably present but when it is the patient is refractory to transfusions (89 282)

Treatment of patients with circulating anticoagulants is most unsatisfactory Large volumes of plasma must be transfused to provide even transient hemostasis (262) Some believe that temporary amelioration of bleeding may result from ACTH or corticosteroid therapy (220 277) but in many cases this regimen has not been helpful (13 112 146 243 262) Fortunately many patients survive what appears to be uncontrollable bleeding Since transfusions apparently increase the anticoagulant titer they should be avoided when possible (4 13) Further investigation in this area might well include a search for some chemical agent which will neutralize the effect of the anticoagulants

Hemophilia in the Female

Genetically the union of a hemophilic male and a female carrier should result in a hemophilic female in a fourth of the births (Fig 2) For a long time the rarity of hemophilia in the female was ascribed to the possibility that the homozygous state for the abnormal gene was lethal for the embryo It seems more likely that the scarcity of cases is due to the infrequency with which a hemophiliac marries a hemophilic carrier In dogs a species in which a disease comparable to human

The fundamental nature of vascular hemophilia is yet to be discerned Singer and Ramot (242) and Matter and associates (164) were impressed by the resemblances between their patients and those described by von Willebrand (292) under the name "pseudohemophilia" For this reason Singer and Ramot named the syndrome "pseudohemophilia type B" Perhaps as Matter *et al* suggest, a single gene determines a step in the synthesis of antihemophilic factor and is needed for the development of the vascular tree This implies that a series of different genes control the sequence of reactions leading to the synthesis of antihemophilic factor Corroborative evidence for this is found in those patients in whom hereditary deficiencies of antihemophilic factor and proaccelerin co-exist (*see* p 164) Another explanation might be that the lesion is fundamentally vascular and that antihemophilic factor is used for hemostasis at the site of the vascular injury at a faster rate than it is synthesized However in patients with severe bleeding because of other hemorrhagic disorders a deficiency of antihemophilic factor is not observed

The prognosis of vascular hemophilia is variable some patients dying in childhood and others surviving into adult life Singer and Ramot believed that when transfusion is necessary, fresh blood is most efficacious, while Schulman *et al* (232) found that they could use freshly frozen plasma effectively It is obvious from the various contradictory statements which have been quoted that this syndrome is just beginning to be unraveled and clarity may be expected only from the study of additional cases

CHRISTMAS DISEASE

Until recently the clinical syndrome of hemophilia was thought to result from a single defect in the coagulative mechanisms With the development of modern technics this unitary concept became untenable. In 1947 Pavlovsky (181) reported that a mixture of the blood of 2 hemophiliacs clotted more rapidly than either blood alone Moreover the transfusion of the blood of 1 patient into the other was as effective as the transfusion of normal blood A similar observation was made by Koller and co workers (131) the blood of patients of the Moena family corrected the defect of patients they thought at the time to be true hemophiliacs. Then in 1952 the same paradox was observed by 3 separate groups of investigators (9 41 231) who divided hemophiliacs into at least 2 groups (*1*) those deficient in classic

and Ramot (242) 14 were in females 3 of them had no familial history of hemorrhage but 4 others had male relatives who were bleeders

The clinical manifestations of this disorder include the usual symptoms of hemorrhagic disease Singer and Ramot s patient typically had a life long history including epistaxes easy bruisability and the development of apparently spontaneous hematomas bleeding from a tongue bite and menorrhagia (212) Postoperative and postpartum hemorrhages (164) and hemarthroses (135) have been described Epistaxis may be so severe that death from exsanguination results (164) In 1 patient the spleen was just palpable (278)

The laboratory differentiation of vascular hemophilia depends upon the co existence of a deficiency of antihemophilic factor and a vascular abnormality In all the cases described the platelet counts have been normal and the platelets have behaved normally with respect to clot retraction and thromboplastin generation However in 1 of the cases reported by Soulier *et al* (251) the platelets were unusually large With few exceptions (164) the bleeding time has been prolonged At the same time in most cases the plasma has been deficient in antihemophilic factor The exceptions have been patients with relatives deficient in this substance (15 164) Because of the presence of a dual defect Schulman and associates (232) have suggested the appropriate name vascular hemophilia" for this disorder

In those instances in which the antihemophilic factor concentration was estimated quantitatively values varying from 3 to 75 per cent of normal have been reported With one exception these concentrations have been considerably higher than those found in most patients with classic hemophilia even of the mild variety Besides the prolonged bleeding time, other evidences of a vascular anomaly have been described Alexander and Goldstein (15) observed an abnormality of the vessels of the nail bed in the mother of 1 of their patients and similar changes were observed in 3 of 7 cases by Matter *et al* (164) The tourniquet test may also give a positive result (164)

The mode of inheritance of vascular hemophilia is not clear In some families the abnormal gene behaves like an autosomal i e nonsex linked mendelian dominant (65 164) Singer and Ramot s (242) patient was the offspring of consanguineous parents but only her elder brother had bleeding phenomena These investigators suggest that the defect in their patient was determined by autosomal recessive genes Since the various patients described do not necessarily have the same disorder these discrepancies are not surprising

been observed in the serum of some carriers or presumed carriers (138 221 222) We have seen 1 woman the daughter of a man with typical but mild hemorrhagic disease who has had minor bleeding phenomena and an abnormally low concentration of Christmas factor in her blood (212)

Christmas disease exists in at least two forms—severe and mild (31)—and, as in the case of classic hemophilia, the intensity of the disorder seems to be the same for all affected members of a family (31) Presumably there is a series of allelic genes each of which is manifested by Christmas disease of a given severity alternately modifying genes may influence the intensity of the defect

The opinion is generally held that Christmas disease is due to a deficiency of Christmas factor This substance is present both in plasma and serum (9 14 41) implying that at most it is only partly consumed during clotting (152) In serum Christmas factor is stable upon prolonged storage at 4 C or in the frozen state and withstands 56 C for 5 minutes (9) It is destroyed by heating serum at 56 C for 10 minutes (41) In common with prothrombin pro-SPCA and Stuart factor Christmas factor is adsorbed from plasma by such substances as barium sulfate and aluminum hydroxide and can then be eluted and partly purified (9) It is associated with the β globulins of plasma (6) is present in Cohn s Fractions III and IV I (289) and can be precipitated between 45 to 50 per cent saturation with ammonium sulfate (289) Partly purified preparations correct the plasma defect of patients with Christmas disease (6 8) However such products also shorten the recalcified clotting time of normal plasma suggesting that they may be contaminated with the ubiquitous Hageman factor (*see* p 152)

The site of synthesis of Christmas factor is not known Biggs *et al* (41) reported that the concentration of this factor was depressed in patients treated with coumarin like drugs an observation repeatedly confirmed This suggests that vitamin K is needed for its synthesis but I am not aware of any reported direct evidence for this Liver disease therefore might be suspected to impair the synthesis of Christmas factor but a decreased concentration of this substance was present in the plasma of only 3 of 17 patients with cirrhosis (99) Curiously the 3 were the only women in this group but the series was too small to be sure that this was significant.

Christmas factor appears to play a key role in the development of thromboplastic activity in shed blood Evidence gained from the thromboplastin generation test suggests that it reacts with antihemo-

antihemophilic factor and (2) those lacking a second substance variously named plasma thromboplastin component, Christmas factor antihemophilic factor B factor IX, and β prothromboplastin Since then innumerable reports have appeared describing the properties of this clotting factor and of Christmas disease the hereditary disease associated with its deficiency. No review can do more than sample the available information

With the separation of Christmas disease from classic hemophilia, attempts were made to determine the relative incidence of the 2 disorders Since the number of individuals in a family who may be tested varies comparison of the number of *patients* with the 2 disorders is not satisfactory Summarizing the number of *families* with one or the other disease in 10 reported series we found that 84 per cent of some 488 families had classic hemophilia and the remaining 16 per cent Christmas disease (215) However these data may give a false impression of the relative frequency of the 2 disorders Biggs and associates (41) found only 1 case of Christmas disease in a systematic study of 35 patients with the hemophilic syndrome In our own experience (215) 80 per cent of 44 families had hemophilia and 20 per cent Christmas disease However among families residing in Cuyahoga County which comprises the metropolitan area of Cleveland only 2 of 32 families had Christmas disease Thus it is possible that Christmas disease is rarer than has been assumed and that undefined influences have led patients with this disorder to the specialized laboratory for diagnosis Christmas disease has been reported from laboratories all over the world and there is no evidence that any racial group is particularly susceptible Cases are seen with comparable frequency in white and Negro families (150 212) The inheritance of Christmas disease follows the same general pattern as that of hemophilia (41 143 221 222 249) The tendency to development of Christmas disease is transmitted as a sex linked recessive trait presumably on the X chromosome As a result Christmas disease is a disorder of males who transmit the abnormal gene to their daughters These women are usually asymptomatic but half their sons will have Christmas disease and half their daughters will carry the trait (Fig 2) In addition sporadic instances have been reported 25 per cent or more of cases appear to arise *de novo* (9 150 219 249 250) As is believed of hemophilia some of these sporadic cases may result from spontaneous mutation Instances of bleeding in the female carriers of Christmas disease have been reported (51 150 221 222) and deficient Christmas factor activity has

In keeping with the greater frequency of mild cases of Christmas disease the over all prognosis is probably better than that of hemo philia but definitive data are not yet available Treatment does not differ in principle from that of hemophilia However Christmas factor is more stable than antihemophilic factor so that bank blood stored for several weeks can be used to advantage (221 262) Bradfield and Case (45) dissent from this view they found that under the ordinary conditions of storage the concentration of Christmas factor in citrated blood decreased sharply within 7 days at 4 C although it was stable in plasma kept at -20 C The amount of blood or plasma needed for therapy appears to be less than that required for the care of hemo philiacs (271) This is consonant with the observation that the defect in Christmas disease may be corrected for 48 hours or longer by the transfusion of a single unit of blood or plasma (6 27 221 290) and that *in vitro* Christmas factor is more stable than antihemophilic factor Purified antihemophilic factor is of course ineffective (7) White and co workers (290) have used repeated transfusions of plasma to prevent hemorrhage in severe Christmas disease but this practice is accom panied by the risks of homologous serum jaundice and perhaps of the development of circulating anticoagulants

Resistance to the beneficial effects of transfusion (12) and the pres ence of circulating anticoagulants (146 250 256) have been described in a few cases of Christmas disease Circulating anticoagulants were found in 2 of 26 patients studied by Lewis and associates (146) In 1 of their patients the anticoagulant titer fell over a period of 6 months during which no transfusions were given only to rise again after an other transfusion this anticoagulant was directed specifically against Christmas factor In another case it was thought to inhibit tissue thromboplastin although the evidence was not clear cut (57) As in the case of hemophilia the effect of the anticoagulant can be demon strated more easily if the patient s plasma is incubated with normal plasma before the mixture is recalcified (57) Treatment of these patients is as might be expected difficult because the Christmas factor in transfused blood is inactivated by the anticoagulant

PLASMA THROMBOPLASTIN ANTECEDENT DEFICIENCY

In 1953 Rosenthal Dreskin and Rosenthal (226) delineated a mild familial hemorrhagic syndrome occurring in both sexes and character ized principally by bleeding after surgical procedures (56 209 225)

philic factor in the early stages of clotting (40) The resulting product then reacts with platelets Whether one of these substances is an enzyme and another its substrate has not been determined However unlike *antihemophilic factor Christmas factor does not disappear during clotting*

The diagnosis of Christmas disease is made in the laboratory The clotting time of whole blood in glass tubes is usually prolonged (41 221 290) *but in mild cases it may be normal (31 41 138)* However the clotting time in silicone coated tubes is virtually always prolonged (160) The thrombin time and prothrombin time are normal so that the defect appears to be localized in the earlier phases of clotting The *serum prothrombic activity is usually higher than normal (41 231)* but prothrombin "consumption may not be abnormal if the defect is mild (31 138 212) However thromboplastin generation is usually abnormal As might be expected the defect measured in this test is *corrected by normal serum but not by normal plasma adsorbed* with aluminum hydroxide since this is deficient in Christmas factor The diagnosis rests ultimately upon the demonstration that the plasma of a patient known to have Christmas disease does not correct the defect in the plasma under study A presumptive diagnosis can be made if the patient s defect can be corrected by normal serum but not by normal adsorbed plasma

The bleeding time and tourniquet tests have almost invariably given normal results (150 231). Combined defects have been reported, and will be discussed later

The clinical picture of Christmas disease is indistinguishable from that of classic hemophilia except that the disease may be mild in relatively more cases (95) The only distinguishing feature of which I am aware is the unusual finding of traumatic rupture of the spleen in a boy the patient survived (160) This complication was also described in an Ohio born Amish boy whose case was reported in 1948 (298) Since Christmas disease occurs with great frequency among the Amish men in Ohio the boy may have had this disease which was not differentiated from hemophilia at that time Bleeding into the nervous system may occur as in hemophilia Schulman and Smith s original patient (231) was first seen because of quadriplegia subsequent to hemorrhage Douglas and MacAlpine (74) *described 4 cases of bleeding into the* nervous system including 1 lethal episode of spontaneous cerebral hemorrhage and 3 instance of compression of peripheral nerves by hematomas

at 25 to 33 per cent saturation with ammonium sulfate (223) or at 20 per cent saturation (209) Rosenthal (223) found maximal activity in Cohn s Fraction IV-1 and only slight activity in Fractions I and III others believe the correct activity lies in Fraction I (107 209) When normal serum was subjected to paper electrophoresis a fraction could be eluted between the β_1 and γ globulin components which contained the corrective fraction (223) Seitz filtration of normal plasma through 50 per cent asbestos pads is reported to result in only slight (223) or marked (209) loss of activity Heating serum at 58 C (209) or 60 C (223) for 10 minutes also impairs its corrective effect

One other observation of great interest has been made by Rosenthal (220) concerning the nature of PTA deficiency The corrective effect of normal plasma increased during storage at −15 C More important the plasma of a patient with a mild abnormality acquired the property of correcting the defect in PTA deficiency when it was stored at −15 C for 4 to 62 days Rosenthal did not see this phenomenon in cases in which the defect was marked nor did Ramot (209) in her cases

The significance of the studies in these interesting patients is not yet clear The differences reported from different laboratories suggest that the various patients may not have the same disorder A second problem to be resolved is whether this syndrome is due to the deficiency of a clotting factor Although a fraction of normal plasma corrects the defect, no evidence that a similar fraction prepared from a patient s plasma lacks the corrective activity has appeared Possibly the effect of normal plasma fractions is not specific A third problem has been posed by the report that the plasma of a patient with PTA deficiency provided by Rosenthal was deficient both in antihemophilic factor and Christmas factor (124) Ingram (114) has also suggested that such patients have multiple partial deficiencies of other clotting factors It is difficult to reconcile this view with the evidence that plasma obtained from patients with hemophilia or Christmas disease corrects the defect of plasma from patients with PTA deficiency and vice versa It is evident that no adequate definition of this group of patients is yet possible

Bleeding in PTA deficient patients is treated by transfusion of blood or plasma Relatively small amounts are said to suffice Rosenthal *et al* (225) demonstrated that the transfusion of plasma shortened the clotting time and in larger amounts corrected the defective serum prothrombic activity in 1 of their patients The plasmas tested included

delivery (85) and dental extractions (226) Epistaxes (222) cutaneous ecchymoses (85 105 225) muscular hematomas (56 173 225) hemarthroses (222 225) bleeding into the brain stem (105) menorrhagia (105) and gingival bleeding (56) have all been reported The clotting time of venous blood is usually slightly prolonged and the prothrombic activity of serum is abnormally high Rosenthal *et al* (226) differentiated this disorder from other coagulative diseases because the abnormal clotting time and serum prothrombic activity were corrected by the blood of patients with hemophilia or Christmas disease They suggested that the plasma of their patients was deficient in yet another clotting factor which they named plasma thromboplastin antecedent (PTA)

Rosenthal and co workers (225) believe that the disorder is transmitted as an autosomal dominant trait with a high degree of penetrance and variable expressivity Thus there is an even chance that a carrier will transmit the disorder to his or her offspring The available data are compatible with this view (56 85 103 209) Sporadic cases have also been observed (209 222)

Only a few reports deal with the frequency of PTA deficiency Among 105 families with hereditary coagulative defects reported by 3 groups (85 219 222) 6 were deficient in this factor These data should not be taken too literally the problems posed by studies of the frequency of coagulative disorders were discussed in relation to Christmas disease

The coagulative defect in these patients is usually mild The results of bleeding time (209 222) and tourniquet tests (105) are normal The clotting time of venous blood is usually only slightly prolonged and may be normal However in nearly all cases the prothrombic activity of serum has been abnormally high (226) and the thromboplastin generation test abnormal (173 209) The defect seems to be localized to the initial stages of clotting the prothrombin time is normal (226) Tests for circulating anticoagulants have been positive in only one case (125a) The abnormal serum prothrombic activity has been corrected by oxalated or citrated plasma or serum stored as long as 2 years in the frozen state (56 223) or at room temperature for 4 months (223) The defect can also be corrected by plasma or serum which has been adsorbed with barium sulfate (223) an eluate of the barium sulfate used to adsorb the plasma also corrects the abnormality

Which fraction of normal plasma corrects the defect is not yet agreed upon The corrective fraction has been said to be precipitated

the children or parents of any of the patients tested The evidence gleaned from these few cases then suggests that Hageman trait is transmitted by autosomal recessive genes (162)

The defect in Hageman trait is apparently in the earliest stages of clotting The thrombin time and prothrombin time are normal Prothrombin consumption is usually impaired (121 135a 210 213 234) and the results of the thromboplastin generation test have been abnormal (121 135a 210 214 234) The abnormality can be corrected by adding minute amounts of plasma from patients with a wide variety of hemorrhagic diseases The plasma of patients under treatment with Dicumarol is also effective (121 160) Paradoxically addition of small amounts of plasma from an individual with Hageman trait is either ineffective (87) or only equivocally effective (224) in correcting the abnormal serum prothrombic activity of blood from patients thought to be deficient in PTA The significance of this observation is not understood

The abnormality in the plasma of patients with Hageman trait can be corrected by adding a fraction of normal plasma The same fraction prepared from individuals with Hageman trait, is without effect The corrective fraction "Hageman factor," is present in plasma obtained from rats horses rabbits dogs sheep mice and guinea pigs but not in duck plasma It can be separated by appropriate technics from fibrinogen prothrombin proaccelerin antihemophilic factor pro SPCA Stuart factor Christmas factor and probably PTA Hageman factor is associated with the globulin fraction of plasma When normal serum is subjected to paper electrophoresis Hageman factor can be eluted from the paper maximally between the β and γ globulins (87 121 214) Other properties of Hageman factor have been summarized in a recent review (214)

The role played by Hageman factor in initiating clotting has not been explained Even small amounts accelerate the clotting of normal blood and increase the rate at which prothrombin disappears during clotting There is evidence that Hageman factor is inert in blood as it is shed and becomes activated during clotting when the blood comes in contact with glass The mechanism by which glass "activates" Hageman factor has been illuminated by experiments with duck plasma which is devoid of Hageman factor activity (212) Duck plasma or the plasma of patients with Hageman trait, inhibits the clot accelerating effect of glass However these plasmas do not inhibit the action of plasma which has previously been treated with glass Thus

1 stored in a refrigerator for a week and then kept frozen for an additional week After a transfusion of 450 ml of plasma 1 of their patients underwent hernioplasty without unusual bleeding

TETARTOHEMOPHILIA A FOURTH PLASMA THROMBOPLASTIN COMPONENT DEFICIENCY

In 1954 Spaet and co workers (258) described the case of a man with a lifelong hemorrhagic disorder which at the time they believed to be unique Studies suggested that his blood was deficient in a factor which could not be identified with the known components of the clotting system They named this factor the fourth plasma thromboplastin component and the disorder characterized by its deficiency tetartohemophilia Through the courtesy of Dr Spaet a group of investigators in several cities restudied this patient recently and it is the consensus that he has mild Christmas disease probably complicated by the presence of a weak circulating anticoagulant (259)

HAGEMAN TRAIT

Hageman trait is an asymptomatic disorder characterized by prolongation of the clotting time The name derives from the first patient studied So far 12 cases have been observed (87 100a 121 135a 210 213 228 238) 6 in males and 6 in females The most remarkable feature is the absence of significant hemorrhagic symptoms despite the usual gamut of injuries operative procedures and deliveries A possible exception is the unverified case described by Sjølin (244a) All but 2 of the cases were discovered by chance The remaining cases were found in a survey of the relatives of 2 of the patients The lack of hemorrhagic symptoms in these patients, whose clotting times are as long or longer than that of most hemophiliacs, illustrates the poverty of our concepts concerning hemostasis

Hageman trait may be a hereditary abnormality In 3 cases (87 121 213) the unusually long clotting time is known to have been present for many years suggesting the lifelong presence of the defect In 2 instances the trait has been familial In 2 cases the patients are sisters but none of many of their relatives tested are affected The 2 sisters are the offspring of a consanguineous marriage In 2 other cases the patients are brother and sister but their parents are unrelated (234) A deficiency of Hageman factor has not been demonstrated in

thromboplastin and calcium It was soon evident that factor V was identical with labile factor (193) plasma accelerator globulin (286) and accelerator factor (78) all of which had been described at about the same time.

Cases of parahemophilia have now been described in about 20 families The disorder occurs in both sexes In many patients the symptoms are mild consisting only of spontaneous epistaxes easy bruisability menorrhagia and at times excessive bleeding after dental extractions or surgical procedures In others the bleeding has been more severe and the patients have had severe hematomas spontaneous gingival bleeding and perhaps as in Owren s first case bleeding into the central nervous system. So far as I can tell, hemarthroses have not been observed The bleeding can be lethal (47) The severity of the illness is roughly related to the severity of the defect measured in the one stage prothrombin time Those with the severest bleeding have had prothrombin times between 70 and 110 seconds (normal values 12–15 sec) Quantitative assays have demonstrated the virtual absence of proaccelerin from the plasma of such patients and 1 to 5 per cent of normal in the plasma of patients with milder bleeding

Parahemophilia is usually familial (47 70 128 150) but sporadic cases have been reported (150 160 176) In some cases quantitative assays have demonstrated that the parents of an affected individual have partial deficiencies of proaccelerin, with or without a mild tendency toward excessive bleeding (145) In others such as Owren s original case (176) a proaccelerin deficiency cannot be demonstrated in either parent although the patients have no demonstrable proaccelerin in their plasma The mother of one young man whom we have studied has a plasma proaccelerin concentration of about 50 per cent of normal his father is unavailable for study (160) Consanguinity has been described in the parents of some patients (128) The variable hereditary pattern makes it impossible to deduce any general rule for the inheritance of parahemophilia

It is noteworthy that in 2 instances congenital cardiovascular defects were associated with parahemophilia (110 145) in 1 family syndactylism (70) and in another case epidermolysis bullosa congenitalis (14) A moderate prothrombin deficiency accompanied the proaccelerin deficiency in 1 patient (70) A simultaneous deficiency of antihemophilic factor proaccelerin may occur (*see* p 164)

Evidence concerning the physiologic role of proaccelerin is conflicting Proaccelerin seems to be a globulin It is precipitated maximally

the plasma of ducks or patients with Hageman trait appears to inhibit the "activation" of Hageman factor by glass

The relative stability and ubiquitousness of Hageman factor suggest that it may contaminate other supposedly purified clotting fractions For example Hageman factor may account for the clot accelerating properties of crude preparations of plasmin which led to the now abandoned view that this enzyme initiates clotting

Finally no hints are available as yet concerning the hemostatic mechanisms which protect these patients from bleeding The explanation of this phenomenon will be of interest

PROTHROMBIN DEFICIENCY

A true congenital deficiency of prothrombin is exceedingly rare Most of the cases originally reported have turned out to be deficiencies of other factors or to be instances of combined deficiencies However Quick and associates (205) have described 3 cases in which moderately severe bleeding phenomena were noted from birth The patients bled after circumcision dental extractions and injuries had hematuria, and bled into muscles and joints though without the development of crippling arthritis The clotting times were slightly prolonged and the prothrombin times greatly prolonged It is of interest that in 1 of them vitamin K_1 therapy partly corrected the defect measured in the prothrombin time Several other patients have been studied in whom prothrombin deficiency was combined with deficiencies of other clotting factors (*see* p 164)

PARAHEMOPHILIA

About 15 years ago evidence began to accumulate that the optimal conversion of prothrombin to thrombin by thromboplastin and calcium required the presence of one or more additional factors Among the more impressive studies was that by Owren (176) of a young woman with a lifelong history of a severe bleeding tendency The one stage prothrombin time was greatly prolonged but the actual concentration of prothrombin was normal However the addition of normal plasma from which the prothrombin had been removed shortened the prothrombin time to normal Owren showed that the patient's plasma lacked a heat labile factor needed for the conversion of prothrombin to thrombin He named the patient's disorder parahemophilia and the missing factor factor V the other 4 being fibrinogen prothrombin,

the laboratory Invariably the one stage prothrombin time will be abnormally long. Among the causes of a prolonged prothrombin time, prothrombin and fibrinogen deficiencies can be readily excluded by specific assays for these substances Deficiencies of prothrombin, pro SPCA and Stuart factor can be excluded if the abnormal prothrombin time is corrected on the addition of fresh plasma from which these substances have been removed by adsorption on such substances as barium sulfate The diagnosis can be confirmed by the inability of aged oxalated plasma or plasma heated at 37 C for 24 hours (295) to shorten the prothrombin time

A number of other tests may give abnormal results in parahemophilia In many reported cases the clotting time of venous blood, whether measured in glass or silicone-coated tubes is prolonged The bleeding time is usually normal, but instances in which it is long have been reported (14 136) The prothrombic activity of serum is usually elevated, implying that the conversion of prothrombin to thrombin is impaired during the course of clotting The results of the thromboplastin generation test are also abnormal. However the abnormality is clearly demonstrable only if the incubation mixture contains the patient s platelets or a suspension of crude cephalin instead of normal platelets since the proaccelerin like activity of normal platelets will partly correct the defect

The blood of 1 patient with congenital parahemophilia contained a circulating anticoagulant which rapidly inactivated either partly purified proaccelerin or the proaccelerin contained in normal plasma (110) The circulating anticoagulant was probably a lipid, and resisted incubation at 56 C for 10 minutes I am unfamiliar with any similar cases

The treatment of parahemophilia like that of other hemorrhagic disorders begins with the avoidance of injury Episodes of bleeding which cannot be checked by local measures may be treated by the transfusion of citrated blood or the plasma separated from such blood. For example Alexander and Goldstein (14) found that 500 ml of citrated blood shortened the abnormally long prothrombin time bleeding time and clotting time of a patient with parahemophilia, but the effect lasted less than 48 hours Although there has been some difference of opinion concerning the stability of proaccelerin in citrated blood under the conditions of storage in a blood bank, Fahey and coworkers (77) have shown that the proaccelerin titer remains high for at least 2 weeks under these circumstances Freshly drawn blood therefore is not needed in treating this disorder

between 33 and 50 per cent saturation with ammonium sulfate (176) and is found particularly in Cohn s Fraction II III (12) It is labile dis appearing rapidly when stored in oxalated plasma and more slowly in citrated plasma (77) The prolonged prothrombin time of plasma of patients with proaccelerin deficiency indicates that this substance is needed for the middle stages of clotting i e the conversion of prothrombin to thrombin by thromboplastin During clotting pro accelerin is probably converted to an active form—accelerin Factor VI or serum accelerator globulin (176 287) The conversion can be initiated *in vitro* by adding thrombin (284) suggesting that a chain reaction may take place during clotting As thrombin evolves it con verts proaccelerin to accelerin this in turn resulting in the production of more thrombin from the prothrombin which is still unconverted

Many schemes have been proposed to explain how proaccelerin or accelerin speed thrombin formation Basic to any hypothesis is the observation that proaccelerin activity is demonstrable in the presence of tissue thromboplastin In spontaneous clotting the proaccelerin or accelerin seems to react after the interaction of antihemophilic factor Christmas factor and platelets (72) Apparently, it exerts its effect on the evolved plasma thromboplastin or upon tissue thromboplastin in association with pro SPCA and calcium (82 178) but the exact order of the reactions involved is in doubt In any case an active compound is formed which converts prothrombin to thrombin Proaccelerin and accelerin activity rapidly disappear during clotting (19 72 73) the mode of their destruction is unknown As a result, the serum of most species is devoid of proaccelerin one exception is bovine serum The destruction of proaccelerin during clotting is slower than normal in the blood of patients with thrombocytopenia (19) hemophilia (19 72) and Christmas disease (72)

The relation of platelets to the pathogenesis of bleeding in para hemophilia is of considerable interest Alexander and Goldstein (14) showed that platelet agglutination is retarded in this disorder Nor mally both platelets and plasma contain proaccelerin like activity whereas in patients with parahemophilia the platelets lack this prop erty (111 145) Experiments of Hjort, Rapaport and Owren (108) indicate that the proaccelerin of normal platelets has been adsorbed from the plasma Platelets from a patient with parahemophilia incu bated with normal plasma acquired the proaccelerin like activity of normal platelets

Usually the diagnosis of parahemophilia is readily established in

hour after blood is drawn only a small amount of the prothrombin remains in the serum However when the conversion of prothrombin to thrombin is impaired the amount of prothrombin remaining in serum is relatively high i e prothrombin consumption is poor

In one group of cases the results of the test for serum prothrombin activity have been normal (20 58 106 119 126 129 177 205 253 264 279) These cases have occurred in both sexes With few exceptions (58 264) the patients have bled from birth or early infancy The pattern of hemorrhage has been similar to that of hemophilia with ready bruising epistaxes gingival bleeding intramuscular hematomas hemarthroses bleeding into the gastrointestinal tract and central nervous system bleeding after dental extractions and in the females menorrhagia Surgical procedures have been tolerated surprisingly well (20 106 147 253) Bleeding is usually not as severe as in hemophilia although fatal cases have been reported (279) In addition to the prolonged prothrombin time the clotting time has been long in many of the patients (20 58 129 177) and the bleeding time in a few (20)

The pattern of heredity is by no means clear Both familial and sporadic cases have been observed In 1 family studied in our laboratory pro SPCA deficiency appeared to be inherited as a recessive trait in which the heterozygous state could be detected (212)

To explain the normal serum prothrombin activity in the presence of a prolonged prothrombin time it has been suggested that these patients lack a factor needed for the action of tissue thromboplastin but not for that of the thromboplastin derived from blood itself (1) In certain of these cases the thromboplastin generation test has given normal results (1 106 126 177 279) supporting this view However this explanation may not prove satisfactory since the clotting time may be prolonged and results of the thromboplastin generation test have actually been abnormal as often as normal (37 58 129 253 268) Finally it is noteworthy that the prolonged prothrombin time in these patients has been corrected by addition of Russell s viper venom (106 119 177 212) which is known to behave as if it contained pro SPCA (120)

Although this disorder seldom threatens life treatment is unsatisfactory The transfusion of fresh or stored blood has been only of the most transient benefit (20) Chevallier *et al* (58) noted that the pro SPCA of normal plasma was inactivated when it was incubated for 6 hours with an equal volume of plasma from 1 of their patients but other cases of circulating anticoagulants have not been reported We

In animals the injection of aminophylline has stimulated an increase in the plasma proaccelerin concentration (109) but aminophylline is without effect in parahemophilia Vitamin K therapy is also ineffective (176) a predictable result since Dicumarol and similar compounds do not alter the proaccelerin concentration

DEFICIENCIES OF SERUM FACTORS PRO SPCA AND STUART FACTOR

A number of patients thought to have congenital prothrombin deficiencies were described in the 1940s Surprisingly the plasma defect in certain of these patients was corrected by adding serum supposedly poor in prothrombin (64 90 123) This discrepancy was clarified by the studies of Alexander *et al* (20) on the nature of the severe clotting defect in a young girl The clotting time of venous blood was normal or slightly prolonged and the bleeding time abnormally long The one-stage prothrombin time was much longer than normal for example 72 seconds compared with 17 seconds for a control plasma The addition of human serum devoid of prothrombin or proaccelerin, shortened the prolonged prothrombin time Presumably then the patient s plasma lacked something present in normal serum which corrected the abnormality measured by the one stage prothrombin time

Just such a substance had been described by a number of investigators who used the usual barrage of descriptive terms co thromboplastin stable factor proconvertin and the precursors of factor VII or of serum prothrombin conversion accelerator (pro SPCA) Alexander *et al* showed that the addition of purified pro SPCA corrected the defect in their patient s plasma and concluded that her plasma was deficient in this factor

Since then other instances have been reported of a congenital hemorrhagic state associated with a long prothrombin time which is corrected by serum These cases do not form a homogeneous group and from the available literature they cannot be classified rationally One classification suggested by recent studies of Ackroyd (1) and of Hougie and co workers (113) would divide these cases according to the results of the prothrombin consumption test In this test the patient s blood is allowed to clot in glass tubes *without the addition of tissue thromboplastin* After a standard interval, the amount of prothrombin remaining in the serum is measured Normally within 1

the basis of the serum prothrombin activity thromboplastin generation, and the reaction to Russell s viper venom fails to account for all the re ported cases For example the patient described by Frick and Hagen (86) has now been restudied by Gonyea and Krivit (92a) Despite the presence of normal serum prothrombin activity appropriate technics showed that the correct diagnosis was Stuart factor deficiency The seemingly unending complexity is illustrated by the case of identical twins described by Biggs (37) The prothrombin time serum prothrom bin activity thromboplastin generation and reaction to Russell s viper venom were all abnormal as in the cases of Stuart defect or Prower defect However serum of the patient with Prower defect (266) cor rected the defect in these twins and vice versa Thus the distinction between the various cases is in a hopeless jumble and awaits some clarifying concept These studies are of great theoretic interest, but their practical application is limited at present to the classification of patients and to an appreciation of the limits of our current diagnostic armamentarium

CONGENITAL AFIBRINOGENEMIA

Perhaps the most extraordinary familial hemorrhagic disease is congenital afibrinogenemia first described by Rabe and Salomon (207) in which no significant amount of fibrinogen is detectable in the blood whatever the technic used—chemical immunologic or electro phoretic The reported cases have recently been reviewed (88 189 252 280) Obviously the blood is incoagulable and a clot does not form when thrombin is added The tissues too are devoid of detecta ble fibrinogen (91) These patients usually bleed from the umbilicus at birth (68 189 252) and have repeated episodes of severe bleeding throughout life They may have cutaneous ecchymoses epistaxes hematomas and hemoptyses and may bleed into the central nervous system (230) Intraperitoneal hemorrhage following rupture of the spleen has been described (101) Hemarthroses are relatively rare (252) Trivial injuries may cause protracted bleeding Death results from blood loss (175) or bleeding into a vital area (230)

Long periods of freedom from bleeding are among the many oddities seen in these patients (21 140 154) Girls who have reached the menarche have had nearly normal menses (140 186) and there have been no exsanguinating hemorrhages such as might be anticipated The disorder points up the role of mechanisms other than coagulation in control of bleeding

were unable to corroborate this observation with the plasma of a patient deficient in pro SPCA in a case to be reported by Hewlett and Battle (105a) Although vitamin K is necessary for proconvertin synthesis it is only occasionally effective in congenital pro SPCA deficiency and then only transiently (129 268)

In summary in a group of cases similar to that reported by Alexander *et al* the prolonged prothrombin time has been ascribed to a deficiency of pro SPCA Tests in these cases demonstrate normal serum prothrombin activity variable thromboplastin generation, and in the few instances tested correction of the abnormality when Russell's viper venom is added

In a second group of cases the results of the test for serum prothrombin activity have been abnormal (26 113 147 266 299) Sporadic and familial cases in both sexes have been described The symptoms do not differ significantly from those of patients in whom serum prothrombin activity is normal In addition to a prolonged prothrombin time the clotting time has usually been long and thromboplastin generation abnormal (113 266) Telfer and co workers (266) differentiated the defect in their patient from pro SPCA deficiency because the prothrombin time was shortened by the plasma of patients under treatment for a short time with phenindione (Dindevan * Danilone †, Hedulin ‡) a substance with coumarin like activity Such plasma is deficient in pro SPCA They believed that their patient lacked another factor and they named her disorder Prower-defect after her surname The reaction to Russell's viper venom in her case was equivocal (37, 266) Similarly Hougie and co workers (113) restudying a case reported earlier (147) observed that the prothrombin time was shortened by the plasma of patients treated briefly with Dicumarol Such plasma too is deficient in pro SPCA Furthermore the plasma defect of Hougie's patient was corrected by addition of plasma from the patient observed by Alexander *et al* (20) implying that the 2 patients lacked different factors Finally Russell's viper venom did not correct the prolonged prothrombin time They concluded that their patient lacked a property other than pro SPCA which they named "Stuart factor" after their patient Restudy of the patient of Crockett *et al* (64) indicated that she had the same defect

Unfortunately the differentiation of these cases into 2 groups on

* Evans Medical Supplies Liverpool England
† Schieffelin & Co New York N Y
‡ Walker Laboratories Inc Mount Vernon N Y

present in normal concentration although thrombocytopenia (54 68 154) and a slight decrease in proaccelerin (144) have been observed Thrombocytopenia has also been associated with congenital hypofibrinogenemia (104 218) The bleeding time varies (280) in some it appears to be normal but bleeding starts again when the patient moves the part which has been tested The result of the tourniquet test is usually normal (88 280) In 1 patient, the plasma albumin level was decreased and the globulin increased but no clinical evidence of hepatic disease was described (280)

Although episodes of bleeding may end without specific treatment transfusion of blood or preferably human fibrinogen is the treatment of choice Because exsanguination or bleeding into a vital area is possible the prognosis is poor To the best of my knowledge the oldest living patient is now 26 years old (101)

CONGENITAL HYPERHEPARINEMIA

Quick and Hussey (204) have recently described the case of a 23 year old woman with a history of abnormal bleeding since the age of 3 Many transfusions had been necessary because of hemorrhage after mastoidectomy after dental extractions and other minor operations and after childbirth The clotting time was prolonged this abnormality was apparently due to a change in the final stages of clotting since the clotting time of a mixture of thrombin and the patient s plasma was abnormally long Since the thrombin time was shortened by the addition of protamine sulfate or toluidine blue substances known to neutralize heparin they believe that the patient has chronic hyperheparinemia Intravenous injection of protamine or toluidine blue did not improve the patient s condition but cortisone and transfusions of freshly frozen plasma were apparently beneficial This case has been reported only in the form of a brief abstract more detailed proof that the blood actually contains heparin or a heparinoid would be desirable Other reported cases of congenital hyperheparinemia are not as convincing as this one since the thrombin time which is altered by minute amounts of heparin has not been prolonged

CONGENITAL THROMBOCYTOPENIA

An interesting study is that by Aldrich and co workers (10) of a family in which the complex of repeated ear infections eczematoid dermatitis bloody diarrhea and purpura appeared in certain individu

The experiment of nature represented by afibrinogenemia provides an opportunity to study the earlier stages of clotting and vascular hemostatic mechanisms unhampered by the formation of fibrin For example Pinniger and Prunty (187) observed that agglutination and fragmentation of platelets occurred in the absence of fibrinogen The clumps of platelets adhered to the walls of a glass tube They thought their observations to be in agreement with the view that platelet thrombi are important in controlling bleeding from small vessels Several groups (21 36 280) have confirmed these observations Alexander *et al* (21) believe that the change in platelets is partly due to elaboration of thrombin in the shed blood Hardisty and Pinniger (101) showed that serotonin is readily released from the platelets in shed blood of a patient with afibrinogenemia in contrast to hemophilic blood in which the release of serotonin is slower than normal (36) they related the relatively mild bleeding in congenital afibrinogenemia to this capacity to release serotonin

Congenital afibrinogenemia is an inherited disease occurring in both sexes and is ordinarily transmitted by mutant autosomal recessive genes Multiple cases within a family may occur (140) Many of the the patients were offspring of consanguineous marriages (21 88 154 187 230 252) The heterozygous state has been undetectable in many cases (187 280) but some of the heterozygous carriers have had hypofibrinogenemia (88 140 154) and some of them have had mild symptoms Isolated cases of congenital hypofibrinogenemia have been described (218) However Graham (95) questions the evidence that heterozygous carriers can be detected

All the observations seem to indicate that congenital afibrinogenemia is probably due to a failure of fibrinogen synthesis rather than to its excessive use or destruction Fibrinogen transfused in the form of blood (46 187) or purified preparations (88 101) can be detected for as long as 17 days although an adequate concentration for hemostasis lasts for a much shorter time Gitlin and Borges (91) found the half life of the infused fibrinogen to be approximately 4 days a value comparable to the half life of radioactive fibrinogen in normal individuals

The diagnosis is easily established The peripheral blood is incoagulable even when thrombin is added the absence of fibrinogen can then be confirmed by several methods When antiserum prepared against human fibrinogen is added to such plasma no fibrinogen (187 285) or only the merest traces can be detected (91) Of course the one stage prothrombin time is infinite Other clotting factors are usually

present in normal concentration although thrombocytopenia (54 68 154) and a slight decrease in proaccelerin (144) have been observed Thrombocytopenia has also been associated with congenital hypo fibrinogenemia (104 218) The bleeding time varies (280) in some it appears to be normal but bleeding starts again when the patient moves the part which has been tested The result of the tourniquet test is usually normal (88 280) In 1 patient the plasma albumin level was decreased and the globulin increased but no clinical evidence of hepatic disease was described (280)

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CONGENITAL THROMBOCYTOPENIA

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als all of them males The syndrome was apparently transmitted by a sex linked recessive gene Studies of the hemostatic mechanisms in 1 affected child revealed a persistent thrombocytopenia which failed to respond to splenectomy The boys bone marrow contained numerous megakaryocytes with little evidence of platelet formation The exist ence of this syndrome has been confirmed by Wolff and Bertuccio (295a) and by Huntley and Dees (113a)

Congenital thrombocytopenia has also been described in association with giant hemangiomas in infancy The available evidence suggests that in this disorder the platelets are sequestered in the tumor Treat ment of the hemangioma results in remission of the thrombocytopenia (93)

Congenital thrombocytopenia at times familial has also been de scribed in the absence of other defects (296) I am unfamiliar with a clear definition of this syndrome

Qualitative defects in platelets have not been considered in the pres ent review

COMBINED DEFECTS

Incredible as it may seem a number of cases have been described in which the blood seems congenitally deficient in more than one clotting factor Thus combined deficiencies of antihemophilic factor and Christmas factor antihemophilic factor and proaccelerin and of prothrombin Christmas factor and "proconvertin" have all been re ported Soulier and Larrieu (250) described the case of a 39 year old man in whom deficiencies of both antihemophilic and Christmas factors were detected This patient had a family history of a bleeding diathesis but the nature of the disorder in his relatives was not ascertained Hill and Speer (107) reported the cases of 2 brothers with a severe bleeding disorder which they attributed to a combined deficiency of the same factors their data however are susceptible to other interpretations Ingram (114) described the same combination in a woman whose father brother 2 sisters and paternal aunts were all bleeders infor mation about her mothers family was not available In addition the patients bleeding time was prolonged and Ingram thought the plate lets to be qualitatively abnormal Verstraete and Vandenbrouckes (281) patient with the same deficiencies had 2 maternal male cousins with classic hemophilia alone

Combined deficiencies of proaccelerin and antihemophilic factor have also been observed in 4 cases all male (116 174 238) Oeri and

associates (174) have described 2 brothers with relatively mild hemorrhagic symptoms in whom defects in both factors were found The same combination was noted by Iversen and Bastrup Madsen (116) in a mentally retarded boy severe bleeding had resulted in hemarthroses and in priapism Our patient (238) has had only mild hemorrhagic manifestations The disorder seems to be due to the inheritance of recessive genes which may not be sex linked since 3 patients were offspring of consanguinous unions (116 174) and the parents of the fourth came from the same small village (238) A sister of 1 patient had hypoproaccelerinemia but the parents and 2 other siblings were normal (116) The mother of our patient had a slight proaccelerin deficiency but *the only other available relative a maternal half brother* was normal It would thus appear that the defects can be so inherited that one or both deficiencies appear It remains to be seen if any relatives will have a deficiency of antihemophilic factor alone

It is noteworthy that antihemophilic factor and proaccelerin have a number of similar properties (*1*) both are labile upon storage or gentle heating particularly in the presence of oxalate (*2*) both disappear rapidly during clotting (*3*) both are poorly adsorbed by barium sulfate and similar agents (*4*) both are adsorbed onto platelets and (*5*) both are only poorly inhibited by sulfhydryl containing reagents (212) The findings would be compatible with the view that certain genes influence the synthesis of both proteins but there are no data to suggest the nature of the genetic influences on these syntheses

In a third type of combined defect, reported by Newcomb and associates (172) the patient s plasma resembles that of individuals under treatment with coumarin like drugs Their patient, an adult woman had had a hemorrhagic tendency since the age of 4 characterized by ecchymoses hematemesis hemoptysis melena hematuria menorrhagia cerebral hemorrhage and bleeding after tonsillectomy It is not clear whether a hereditary defect was present, although several relatives had histories suggestive of a bleeding tendency In addition to the hematologic abnormalities a number of bizarre manifestations including arthralgia splenomegaly and lymphadenopathy were present The prothrombin time was greatly prolonged an abnormality shown to be due to simultaneous deficiencies of prothrombin pro SPCA and Stuart factor (172) a deficiency of Christmas factor was also present (37) All these changes resemble those in patients treated with coumarin like drugs we have also seen comparable abnormalities in a patient apparently sensitive to relatively small doses of aspirin

The possibility of self administration of drugs was excluded by Newcomb et al Massive doses of vitamin K_1 were of the most transient benefit but transfusion of 500 ml of fresh plasma resulted in a remission lasting 1 or 2 weeks On the other hand fresh serum had only a temporary effect Newcomb et al suggested that the patient might lack some substance required for the synthesis of all of the deficient factors Similar cases may have been observed elsewhere (274)

This summary does not encompass the various combinations of coagulative defects which have been reported Without laboring the point it is evident that one must guard against dogmatic acceptance of the relatively simple genetic concepts which have dominated our thinking about hemorrhagic disease

L ENVOI

A common human failing is to hope for a return to an earlier day in which peace prosperity the various homely virtues and a simplified concept of coagulation all reigned serenely In our more lucid moments the obstacles in our way to these goals are clear enough Perhaps a realistic view of the future would include an increasing appreciation of the biochemistry and biophysics of clotting more rational therapeutics and the emergence of a new strain of clotologists deficient in the enzymes which are responsible for terminology

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Etiology and Pathogenesis of Glomerulonephritis*

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MANY clinicians and pathologists have a rather clear concept of the etiology and pathogenesis of acute glomerulonephritis However careful analysis of published reports on nephritis both in man and in the experimental animal reveal many gaps in our knowledge as well as many facts which are difficult to reconcile with present theories of the causation of nephritis

There is general agreement that acute glomerulonephritis is not a result of direct bacterial invasion of renal tissue On the other hand it is generally accepted that the syndrome is etiologically related to an antecedent infection of the respiratory tract or of the skin caused chiefly by the hemolytic streptococcus Because nephritis follows rather than accompanies this infection it seems likely that the renal reaction is not directly due to bacterial toxins or bacterial metabolites In view of the latent period of 1 to 3 weeks between the "triggering" infection and the onset of nephritis it has been proposed that this renal inflammatory reaction represents a hypersensitivity to bacterial protein or an autoimmune response to renal components altered by reaction with bacterial products

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We propose to describe the natural history of glomerulonephritis and to emphasize some of the difficulties in establishing a clear cut diagnosis of the disease to describe and discuss the epidemiologic evidence relating antecedent streptococcal infection to glomerulonephritis to summarize the methods that have been employed to produce experimental nephritis and to discuss briefly the resulting experimental syndromes and their applicability to the study of human nephritis and to emphasize the potential value of newly developed pathologic technics in the diagnosis and in etiologic investigations of nephritis

NATURAL HISTORY

Classically the initial attack of acute glomerulonephritis begins with the appearance of dark brown or smoky urine often accompanied by swollen ankles or hands and swelling of the face around the eyes These signs characteristically appear 1 to 3 weeks after a respiratory infection or occasionally following infection of the skin They may be accompanied by vague lumbar soreness pain in the abdomen or groins and anorexia vomiting and oliguria These symptoms and signs prompt the patient to seek medical attention and the demonstration of proteinuria, hematuria red blood cell casts and frequently hypertension establish the diagnosis

Usually the disease is not severe and clinical improvement is noted within a few days or weeks The mortality rate of hospitalized patients during the acute phase is less than 5 per cent (11) In the absence of hypertension or edema death rarely occurs Symptomatically most patients are usually well within several weeks but the urine may show moderate numbers of red blood cells and albumin for several weeks or months Most patients recover completely and no evidence of permanent damage to the kidney remains In 5 to 15 per cent, chronic glomerulonephritis develops, according to Earle (11) In such patients albuminuria may at first be the only manifestation of continuing renal disease but later signs of decreased renal function appear

The factor responsible for the initiation of an attack of acute glomerulonephritis seems to be infection with group A streptococci This relation seems to be well established although other bacterial agents have been incriminated in a few cases Since infections with group A streptococci involve the respiratory tract and less commonly the skin the patient with nephritis usually has a history of sore throat,

scarlet fever or skin infection The signs of nephritis usually develop after recovery from the acute phase of the bacterial infection)

PROBLEMS PRESENTED BY VARIATIONS IN DISEASE

As a physician s experience with Bright s disease broadens his original concept of the uniformity of glomerulonephritis is modified He becomes aware of the wide variations in the intensity of the disease varying from the patient with microscopic hematuria detected only as a result of the physician s diligence in obtaining and examining a urine specimen to the patient hospitalized with hypertension convulsions anuria and azotemia Instead of the sudden onset, 1 to 3 weeks after a respiratory infection of well delineated symptoms and signs that persist for several weeks and then subside completely the physician also observes patients in whom the onset of renal signs and symptoms is insidious, patients with marked proteinuria and edema and patients in whom persistent or progressive renal disease develops Thus it becomes evident that nephritis is a complex disease process of man of diverse clinical nature and etiology

Clinical Features

One of the intriguing features of acute glomerulonephritis is the factor responsible for the initiation of the disease It has been recognized for years that scarlet fever respiratory infections including tonsillitis or pharyngitis skin infections otitis media sinusitis and pneumonia may precede nephritis) For example Winkenwerder and co workers (96) found that 71 per cent of 78 patients with nephritis had recently experienced an attack of sore throat tonsillitis pharyngitis sinusitis or otitis media A diagnosis of scarlet fever was established in only 2 per cent In addition in 7 per cent pneumonia preceded the nephritis and in 4 per cent an attack of rheumatic fever appeared to be related to the kidney complication In this group, 85 per cent of those who did not have a history of a preceding acute respiratory infection or who had a chronic infection showed progressive renal disease In contrast the course in patients in whom onset was preceded or accompanied by an acute infection with constitutional symptoms was almost invariably favorable) In the pediatric age group the relation of the preceding infection to the course of the disease appears even more clear cut) Aldrich (1) found that chronic

renal disease did not develop among 400 patients with hemorrhagic nephritis following an acute *respiratory infection* In addition it is common experience to see patients with chronic nephritis without a history of an acute episode These clinical observations suggest that signs of nephritis may be produced by at least two different mechanisms (1) the disease usually follows an acute infection of the respiratory tract and is associated with an extremely favorable prognosis and (2) the acute episode of nephritis is either related to chronic *infection or to no infection whatsoever In the latter group* signs of chronic renal disease frequently develop In the present discussion

TABLE 1 —RELATION OF ACUTE GLOMERULONEPHRITIS TO ACUTE PHASE HEMATURIA IN TYPE 12 INFECTIONS (85)

ACUTE-PHASE HEMATURIA	NUMBER OF PATIENTS	NUMBER WITH NEPHRITIS
Present	44	12
Absent	140	9

primary consideration will be given to that form of nephritis which *follows* an acute respiratory infection

During the acute phase of glomerulonephritis erythrocytes and usually *erythrocyte casts* can be demonstrated in the urine However it does not follow that all patients whose urine contains erythrocytes actually have glomerulonephritis Red cells may appear in the urine because of pathologic lesions in the urethra bladder ureters and renal pelves In addition hematuria usually mild is associated with *the acute phase of febrile disease caused by viruses or bacteria* as well as with other illnesses such as cardiac failure Erythrocytes derived from the glomerulus frequently are distorted so that occasionally the source of bleeding can be ascertained by careful microscopic examination of the urine

Because the microscopic urinalysis has been routinely delegated to inexperienced technicians the physician knows little about the nature *and interpretation of hematuria (66) Not only* is the precise identification of each cellular component important but some quantitation is required for proper interpretation Recognition of distorted erythrocytes and stroma erythrocyte casts or casts stained with hemoglobin enables the physician to judge reliably whether glomerulonephritis is present Quantitative studies have been used only on a limited scale probably because of the difficulties in performing Addis counts

The fact that moderate transient hematuria has been observed in a few patients with scarlet fever (44) has created doubt about the value of quantitative erythrocyte enumeration in the diagnosis of acute nephritis

The adoption of a modified Addis count whereby a *fresh* concentrated urine specimen is studied directly in a hemocytometer without

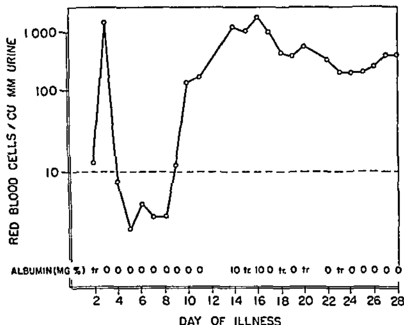


FIG 1 —Pattern of hematuria and albuminuria in a patient with type 12 streptococcal infection

prior centrifugation makes possible the examination of large numbers of specimens Experience with this technic suggests that most normal subjects excrete less than 10 erythrocytes per cubic millimeter (79) Those whose urine contains more than 10 erythrocytes usually have had recent experience with nephritogenic streptococci or show some other cause for the hematuria (79) In patients with respiratory infection including streptococcal disease and scarlet fever the daily enumeration of excreted erythrocytes revealed that the number of cells increases during the febrile period (85) In the patients in whom signs of glomerulonephritis subsequently develop hematuria is especially

likely to appear during the first few days of the respiratory illness (Table 1) These observations which require confirmation indicate immediate damage of the glomerulus by some product of the streptococcus which does not depend on an immune mechanism However the initial hematuria disappears only to return at the time of the onset of nephritic symptoms (Fig 1) The latter phenomenon may be caused by a different mechanism It should be emphasized that the hematuria observed in these patients is not transitory but persists for several days or weeks

It has been said that the diagnosis of acute glomerulonephritis cannot be made in the absence of albuminuria (2) This appears to be a sound observation when based on studies of patients with edema or hypertension but in their absence albuminuria may be slight or nonexistent during the period of hematuria (Fig 1) Indeed persistent severe albuminuria which begins during an acute infection may indicate chronic renal disease For example during a food borne epidemic of type 5 streptococcal disease only 1 of 100 patients observed showed signs of nephritis (10) This patient had hematuria erythrocyte casts and large amounts of albumin in the absence of hypertension or edema It was easy to establish that this was an example of an acute exacerbation of chronic nephritis the patient had a history of repeated episodes of acute hematuria and albuminuria after tonsillitis The emphasis on albuminuria as an important diagnostic criterion may possibly be responsible for much of the confusion concerning the natural history of acute nephritis

ETIOLOGY BACTERIOLOGIC AND SEROLOGIC STUDIES

The facts that scarlet fever may precede nephritis (89) that group A streptococci may be isolated from many patients after symptoms of nephritis develop (96) and that the antistreptolysin titer of a serum specimen obtained during the first few weeks of illness is usually high (40 75) attest that acute glomerulonephritis is a complication of a streptococcal infection The studies of Seegal and co workers (76) showed that the number of hospital admissions for acute rheumatic fever a known complication of streptococcal disease bore no relation to the number of admissions for acute nephritis This observation, as well as the epidemic occurrence of nephritis and the variable attack rate following proved streptococcal infections indicated that strains of group A streptococci might vary in their ability to produce renal disease

The first method used to determine whether strains of streptococci varied in their nephritogenic capacities was the serologic classification of organisms isolated from the oropharynx of patients with acute glomerulonephritis (63) With this technic most strains obtained from sporadic cases of nephritis were found to belong to type 12 (62) Furthermore type 12 was frequently isolated during epidemics of nephritis from both patients and contacts (64 79) The association of type 12 streptococci with nephritis has been recorded throughout the United States (62) and in Canada (64) Australia (50) England (94) Chile (77) and Japan (35) In one epidemic in Minnesota (33) a new serologic type named Red Lake (86) was recognized the same strain has been isolated from a patient in Chile (77) Type 4 streptococci have occasionally been isolated from sporadic cases in this country (62) and have been identified in cultures from patients and their contacts in epidemics in Honolulu (61) and Japan (35) Type 25 has also been isolated from a few cases of nephritis in this country (63) and in Denmark (3) Other serologic types have occasionally been isolated from patients with nephritis (35 23, 77, 93)

This is not an entirely satisfactory method of establishing the nephritogenic character of various types of group A streptococci since by the time signs of nephritis develop (on the average about 10 days after the respiratory infection) few organisms may be present in the nasopharynx and in some cases the isolated organism may represent an old infection or a recent cross infection A more difficult problem for interpretation is presented by the isolation of group A streptococci which do not belong to a known nephritogenic type In such cases it is safer to suspect that the current illness is an exacerbation of chronic disease In the presence of epidemics of streptococcal infections due to any serologic type individuals with chronic nephritis may be expected to experience exacerbations As an example an acute exacerbation with a type 5 streptococcal infection developed in a patient with chronic nephritis (10) but no nephritis occurred among the other 99 men with similar infections

A second and more accurate method for establishing the relative role of various serologic types in the production of nephritis is the *study of streptococcal infections throughout the period in which the* renal complication develops Under these circumstances the original infecting strain can be identified before the onset of nephritis and regular cultures and clinical and laboratory observations will assure that a cross infection with a new serologic type did not occur In one

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such study four serologic types were responsible for an epidemic of streptococcal infections it was clear that type 12 was nephritogenic, whereas types 3 6 and 19 were not followed by acute nephritis (85) In this epidemic severe hematuria occurred in 2 patients early in the streptococcal infections which were caused by types 6 and 19 respectively the hematuria and albuminuria were observed early and persisted for many weeks indicating an exacerbation of mild chronic

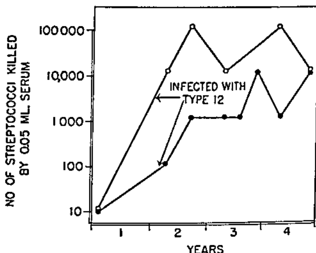


FIG 2 —Persistence of antibody to type 12 streptococcus in 2 patients after infection

nephritis Neither patient gave a history of a preceding attack of nephritis

A third method not used widely is the determination of type specific antibodies in serums or whole blood obtained from patients who have recovered from an attack of acute nephritis Type specific antibodies which persist for several years develop in individuals infected with group A streptococci (Fig 2) Thus many patients with nephritis should show antibodies against one of the nephritogenic strains were these strains etiologically related to the disease Patients in the Cleveland area have been studied by this method (57) Most of those who recovered from an acute attack of hemorrhagic nephritis showed antibody against type 12 streptococci whereas serums from patients with nephrosis and chronic nephritis revealed no antibody as measured in the bacteriostatic test Another study of serums collected in the Chicago area from patients with acute nephritis failed

to show antibody against type 12 suggesting that some other type was responsible for the complication (78) Recently Braude *et al* (5) by the use of bacteriostatic mouse protection and complement fixation technics demonstrated antibodies against the Red Lake strain in individuals with nephritis in Minnesota and antibodies against type 12 in serums from patients with nephritis in Texas. Since antibodies to specific serologic types may persist for several years an increase in type specific antibody against a nephritogenic organism is the best indication of its etiologic significance

Demonstration of the presence of type specific protective antibody in the blood of patients who have recovered from a classic attack of acute glomerulonephritis may help to explain certain clinical observations on recurrences of this disease The presence of this antibody indicates immunity against infection with a specific type (90). It has been frequently said (42) and occasionally recorded (40) that the patient who completely recovers from acute glomerulonephritis rarely if ever suffers a second attack. Knowing that only a few serologic types are able to produce nephritis it would be surprising if many patients became infected with more than one nephritogenic type Certainly the individual who has experienced one attack of nephritis should be immune against a second attack due to the serologic type responsible for the original attack Thus freedom from recurrences in patients who recover from an attack of nephritis may be due to type specific immunity or to the statistical improbability of acquiring a second infection with a nephritogenic type. In addition the product of nephritogenic streptococci which may initiate nephritis may be antigenic so that in a second infection the nephritogenic substance would be neutralized *in vivo*

It is more difficult to explain recurrences in patients with chronic nephritis Exacerbations are said to occur after a short latent period (11 74) and this has been used as evidence in support of an immunologic mechanism in the causation of nephritis (14) While it is true that many exacerbations appear to be initiated by streptococcal infections (74) it might be asked why a second attack develops in these patients whereas in the patient who has recovered completely there is no recurrence after a streptococcal infection (74). Perhaps in the former patients the recurrences are nonspecific since there is evidence that recurrences do follow nonstreptococcal infections (74) Study of the serologic types of streptococci isolated from patients with chronic nephritis experiencing recurrences would help to clarify this problem

Preliminary studies indicate that the serums from patients with chronic nephritis do not contain type specific antibodies against nephritogenic streptococci (60) These data suggest that the pathogenetic mechanisms in exacerbations of chronic nephritis differ from those involved in the initial acute attack of glomerulonephritis

Perhaps one of the most puzzling features of acute glomerulonephritis is the fact that many patients exhibiting classic signs of the disease fail to show β hemolytic streptococci on culture This fact alone has probably led to more confusion concerning etiology than any other observation If all cases of initial attacks of acute nephritis are caused by nephritogenic streptococci why is the organism difficult to isolate? The situation is somewhat reminiscent of the difficulty in isolating streptococci from cases of rheumatic fever in which only 50 to 70 per cent of the oropharyngeal cultures show group A streptococci (6) Occasionally β hemolytic streptococci are isolated from only 15 per cent of patients with rheumatic fever (51) Bacteriologic data (59) suggest that the number of streptococci decreases rather rapidly following an acute streptococcal infection so that a single oropharyngeal culture cannot be considered sufficient to exclude the possibility of infection with these organisms In addition certain biologic characteristics of nephritogenic streptococci may be responsible for these variable bacteriologic results Colonies of the organism on sheep blood agar are not typically large and mucoid but are small and white (58) Even more striking is the absence of wide zones of β hemolysis In a recent outbreak resulting in several thousand cases of nephritis in Japan (35) type 12 streptococcus was found to produce such small zones of hemolysis that many of the colonies could be overlooked by the average bacteriologist This is not a new observation as shown by earlier studies of streptococcal infection epidemics caused by nonhemolytic variants of group A streptococci In each instance, type 12 streptococci were involved In 1941 Coburn and Pauli (9) described a nonhemolytic type 12 streptococcus responsible for an epidemic among infants Francis (17) reported an epidemic of type 12 infections on a plastic surgery ward cultures of the organism obtained from those patients failed to produce β hemolysis on horse rabbit, or human blood agar He has isolated a nonhemolytic type 25 streptococcus Apparently therefore failure to demonstrate typical β hemolytic colonies in cultures obtained from patients with glomerulonephritis may be due to failure to recognize nonhemolytic or poorly hemolytic colonies It is possible that infection with such strains may stimulate

little antistreptolysin production since *in vitro* evidence suggests that production of streptolysin O is slight

Because of the difficulties involved in bacteriologic studies and the lack of careful correlation of bacteriologic data with clinical and pathologic findings it has not been possible to evaluate reports claiming that other microorganisms produce acute glomerulonephritis The pneumococcus for example has long been implicated as a cause of acute glomerulonephritis (52 56 73) It is true that excretion of albumin and red blood cells may be increased in cases of nephritis attributed to pneumococcal infection but hypertension and edema are not common (73) This observation alone might indicate that the nephritis in some patients with pneumonia may be an exacerbation of chronic nephritis In the most detailed study Seegal (73) reported 7 cases of nephritis among 1 004 patients with lobar pneumonia The prominent features included marked albuminuria in the absence of significant hypertension anemia was found in 6 patients edema in 3 patients only Throat cultures for β hemolytic streptococci were negative in 2 of the 7 cases studied and in 1 of these the antistreptolysin titer was normal It is apparent, therefore that no adequate studies were done to exclude infection with nephritogenic streptococci in addition the clinical features of the illnesses were somewhat unusual Final conclusions concerning the production of initial attacks of acute glomerulonephritis by agents other than the streptococcus must await further study

We have presented only selected data bearing on the natural history of nephritis and the precipitating factors in order to emphasize some of the problems encountered by the investigator interested in pathogenesis and etiology Since the response of the kidney to noxious stimuli is limited the clinician can only suggest that nephritis is produced by several different mechanisms With the more precise bacteriologic and serologic methods now available it should be possible to define various forms of renal disease Much of the present confusion arises from lack of knowledge of the etiologic agent and of the natural course of the disease

PATHOLOGY

Many of our concepts of nephritis have come from the extensive contributions of pathologists The lesion in the kidney is considered to be limited to the glomerulus There is proliferation of the endo-

thelium infiltration of the glomeruli with polymorphonuclear leukocytes escape of erythrocytes into the capsular space and thickening of the basement membrane There may be obliteration of the capsular space and the development of crescents The latter Ehrlich (12) considers to represent subacute glomerulonephritis) Although these pathologic features have been frequently described it is obvious from the literature that the evolution of the cellular changes has not been precisely defined and this is especially true of those patients in whom nephritis is known to develop after an infection with nephritogenic streptococci Furthermore according to Bell (2) the changes in the glomerulus may be seen during the course of several infectious diseases and therefore may not be specific Since the course of illness following infection with nephritogenic streptococci may be different from that observed after nonstreptococcal illnesses the evolution of the renal changes may likewise be different By employing modern bacteriologic and serologic technics together with the recently developed technic of renal biopsy it should soon be possible to define the course of acute streptococcal glomerulonephritis In addition since streptococcal infections caused by nephritogenic or by presumably non nephritogenic organisms may precipitate acute exacerbations of chronic nephritis biopsy specimens obtained early in the disease should help in the recognition of chronic nephritis Consider for example the case of a 12 year old boy in whom hypertension edema and hematuria developed after a type of 12 streptococcal infection (88) A renal biopsy was obtained 10 days after the onset of the sore throat. There were large numbers of leukocytes in the glomerulus marked proliferation of the endothelium and glomerular ischemia, and in addition, glomerular hyalinization tubular atrophy and interstitial infiltration The latter Vernier (88) interpreted as evidence of chronic changes which must have existed before the onset of the acute disease Thus this patient may have had two diseases acute hemorrhagic nephritis due to type 12 streptococci superimposed on chronic glomerulonephritis Further serial biopsy studies designed to include specimens taken within a few days of the onset of illness should permit a description of the sequence of histopathologic changes typical of acute nephritis Correlation of these findings with the clinical disease and with bacteriologic and serologic data should make possible a precise differentiation of acute nephritis from chronic nephritis and from other forms of renal disease Such an integrated study may possibly also provide some insight into the pathogenesis of glomerulonephritis

EXPERIMENTAL NEPHRITIS

For many years investigations of the etiology of nephritis have been based on the concept that the pathogenesis of this disease is a manifestation of hypersensitivity Since acute glomerulonephritis as it occurs in man is not a natural disease of animals the investigator has used in animal studies several laboratory models resembling the human disease but not duplicating it The technics have consisted of the injection into a variety of animals of heterologous protein, nephrotoxic serums, mixtures of bacteria and homologous renal tissue or bacteria, specifically hemolytic streptococci alone The clinical and pathologic manifestations after these procedures have been compared with the corresponding features of human renal disease Since the manifestations of these experimental nephritides have been described in detail in several reviews (13 43 67 70) only their major characteristics will be summarized here

Heterologous Protein Nephritis

Glomerulonephritis and cardiovascular lesions have been reported in rabbits in which serum sickness was induced by administration of horse serum (46 67 97) purified serum albumin (18 24 97) or serum γ-globulin (18 24, 50 97) A single large dose or several small doses of these antigens resulted, after a latent period of 8 to 10 days in the appearance of fever proteinuria hematuria, and cylindruria Histologic examination of the rabbit tissues obtained at various intervals after induction of the hypersensitivity reaction revealed, in the early acute state glomerular enlargement with swelling and proliferation of endothelial and epithelial cells of the tuft as well as thickening of the glomerular basement membrane Occasionally at this time but usually later in the course of the inflammatory reaction, adhesions between glomerular tuft and capsule as well as glomerular crescents appeared Some (67) reported the accumulation of polymorphonuclear leukocytes in the glomeruli but this was not a consistent finding Tubular lesions were uncommon, and when found were minimal in degree (18 24 46 50 97) The lesions consisted of patchy hyaline or granular changes in tubular cells and, sometimes atrophy of tubular cells and dilatation of tubular spaces Some tubules contained sizable amounts of amorphous staining material. Excepting those animals that succumbed to anaphylactic shock after repeated injections of antigen, most survived

thelium infiltration of the glomeruli with polymorphonuclear leukocytes escape of erythrocytes into the capsular space and thickening of the basement membrane There may be obliteration of the capsular space and the development of crescents The latter Ehrlich (12) considers to represent subacute glomerulonephritis) Although these pathologic features have been frequently described it is obvious from the literature that the evolution of the cellular changes has not been precisely defined and this is especially true of those patients in whom nephritis is known to develop after an infection with nephritogenic streptococci Furthermore according to Bell (2) the changes in the glomerulus may be seen during the course of several infectious diseases and therefore may not be specific Since the course of illness following infection with nephritogenic streptococci may be different from that observed after nonstreptococcal illnesses the evolution of the renal changes may likewise be different By employing modern bacteriologic and serologic technics together with the recently developed technic of renal biopsy it should soon be possible to define the course of acute streptococcal glomerulonephritis In addition since streptococcal infections caused by nephritogenic or by presumably non nephritogenic organisms may precipitate acute exacerbations of chronic nephritis, biopsy specimens obtained early in the disease should help in the recognition of chronic nephritis Consider for example the case of a 12 year old boy in whom hypertension edema, and hematuria developed after a type of 12 streptococcal infection (88) A renal biopsy was obtained 10 days after the onset of the sore throat There were large numbers of leukocytes in the glomerulus marked proliferation of the endothelium and glomerular ischemia and in addition glomerular hyalinization tubular atrophy and interstitial infiltration The latter Vernier (88) interpreted as evidence of chronic changes which must have existed before the onset of the acute disease Thus this patient may have had two diseases acute hemorrhagic nephritis due to type 12 streptococci superimposed on chronic glomerulonephritis Further serial biopsy studies designed to include specimens taken within a few days of the onset of illness should permit a description of the sequence of histopathologic changes typical of acute nephritis Correlation of these findings with the clinical disease and with bacteriologic and serologic data should make possible a precise differentiation of acute nephritis from chronic nephritis and from other forms of renal disease Such an integrated study may possibly also provide some insight into the pathogenesis of glomerulonephritis

foreign protein during the period when antigen is rapidly disappearing from the circulation and renal lesions are appearing offers additional suggestive evidence that these nephritic lesions are the result of antigen-antibody reaction (69 91) A similar fall in serum complement titer in human glomerulonephritis has been reported (15 92)

The hypothesis that glomerulonephritis is related to the reaction of host antibody to injected foreign protein is further supported by the observation that suppression of antibody formation after injection of antigen by treatment with roentgen rays or nitrogen mustard prevents the appearance of renal and cardiovascular lesions (69 91) Cortisone and ACTH also apparently suppress the development of glomerulonephritic and cardiovascular lesions by interference with antibody formation (20) Other workers using ACTH have reported that these lesions can be suppressed without interference with antibody formation indicating that the hormones may function by changing tissue reactive capacity (67 91) It is noteworthy that ACTH and cortisone have been reported to be without effect on the course of nephrotoxic renal disease in rats (22, 34 71)

Nephrotoxic Renal Disease

The production of nephritis in animals with nephrotoxic or antikidney serums has been extensively studied as an experimental model of human renal disease The nephrotoxic serum reagent is prepared by the injection as antigen of homogenized kidney tissue or isolated fractions of kidney tissue from one animal into another animal species To produce nephrotoxic renal disease, the antikidney serum is then injected into the animal species from which the kidney tissue was originally obtained Nephritis has been produced by this technic in rats (13 27 28 39 70 81–83) rabbits (13 32 70 80) and dogs (4 16 70, 72) with nephrotoxic serum made in ducks chickens and rabbits Although the renal disease produced in these animals with different nephrotoxic serums has many similarities certain differences have been discerned

Clinical Manifestations.—In the rat, a form of nephritis characterized by severe proteinuria, hypoproteinemia hypercholesterolemia and edema has been described (13 27 28 39 70 81–85) The close similarity of the clinical and urinary findings of this disease with the nephrotic syndrome as it occurs in children has been pointed out (27) These manifestations appear almost immediately after the injection of

this hypersensitivity reaction Relatively complete healing of the glomerular lesion was found in animals followed for several months (18 24, 50 97) Azotemia occurred in a small proportion of animals receiving injections of antigen over long periods of time (46)

In addition to the renal lesions in foreign protein nephritis myocarditis endocarditis generalized arteritis accompanied by lymph node hyperplasia, and granulomas occur with variable frequency (18 19 24 50) The nature and incidence of the lesions produced by heterologous protein injections vary with the nature and dosage of the antigen and the frequency and duration of its administration (19 24 25) Glomerular lesions are reported to appear earlier after the injection of γ globulin than of albumin (24) Arteritis is more likely to be a prominent pathologic feature of prolonged sensitization where as glomerulonephritis is more frequently seen shortly after a single or relatively few doses of either antigen (19 25) A significant degree of unpredictable variation in the production of glomerulonephritis in individual animals has also been emphasized (67)

Concurrent immunologic and histologic studies in heterologous protein hypersensitivities have shown that the lesions of glomerulonephritis after a single intravenous injection of antigen appear during the last stages of antigen elimination and are maximal just before the appearance of free circulating antibody (18 21 24) After free antibody appears the renal lesions as well as the cardiac lesions regress (18 21 24) These findings indicate that renal and cardiovascular lesions occur at a time when antigen and antibody may both be present in tissues and extracellular fluid (18 21 24) and are consistent with the hypothesis that the lesions result from antigen-antibody combination

Efforts have been made to demonstrate histochemically the localization of antibody in renal lesions in experimental animals given foreign protein (47) and in sections of human kidney obtained at autopsy from patients with nephrosis and several types of nephritis (48 87) Gamma globulins have been shown to concentrate in damaged glomeruli in kidney sections from cases of nephrosis and in several types of nephritis but proof that this is specific antibody has not yet been obtained

Complement fixation is a well known feature of *in vitro* antigen-antibody reaction and there is evidence that it occurs *in vivo* after the reinjection of antigens into specifically sensitized animals (84) The demonstration that serum complement decreases in animals given

that the variations noted with different types or amounts of serum are quantitative (26 27) The clinical disease in rabbits and dogs appears to resemble glomerulonephritis (13 31 32 70) It is possible that the pattern of host response is a characteristic of a given animal species

Evidence has been accumulated that glomerular tissue (36 37 70 83) and more specifically glomerular basement membrane (36 37) contains the nephrotoxic antigen Glomerular basement membrane produces more potent nephrotoxic serum when compared with other kidney components on a weight basis (37) Glomeruli absorb nephrotoxic activity from antikidney serums (37 83) Radioautography and fluorescence microscopy using antikidney or antiglomerulus serums with radioactive (55) or fluorescein (29 49) labels demonstrate the localization of labeled antibodies in glomeruli particularly in glomerular basement membrane in tissue sections from treated animals The latter evidence may indicate the participating antigen and the site of reaction of nephrotoxic serums The ability of placental tissue to stimulate the production of nephrotoxic serum (70) probably results from the presence in placenta of basement membrane material antigenically related to glomerular basement membrane The localization of components of labeled antiplacenta serums in glomerular basement membranes (70) is evidence for this antigenic relation

In general, attempts to measure nephrotoxic antibody in nephrotoxic serums (38 68) and to achieve a quantitative correlation between the amount of this antibody in a given serum and the severity of nephritis produced by it have been unsuccessful. Inability to obtain more than a rough correlation by precipitation complement fixation or hemagglutination is probably due to failure to identify and isolate the specific antigen responsible for nephrotoxicity

It has been suggested that the lesion of nephrotoxic nephritis is the result of the union of specific antibody in injected antikidney serum with homologous antigen in renal tissue cells (13 43) This hypothesis is supported by the demonstration with fluorescein labeled chicken antiserum against rabbit antibody that injected rabbit antibody against rat kidney localizes in the glomeruli (49) It has been proposed that in the rabbit nephrotoxic nephritis produced with duck antikidney serum, in view of the latent period consists of two phases in the first, injected antikidney antibody in duck serum is bound by rabbit kidney in the second antibody formed by the rabbit against duck globulin reacts with the bound duck antikidney antibody globulin and causes the nephritic inflammatory reaction (31 32) The acceleration of

rabbit antirat kidney serums Hematuria and hypertension are uncommon and transient (27 39 70) In many instances the disease becomes chronic and progresses to uremia and death (28 39 70 81 82)

It has become increasingly apparent that the severity as well as the qualitative variations in several of the clinicopathologic features of nephrotoxic nephritis in the rat depend upon the dosage potency source and method of administration of the antirat kidney serums and on the age sex and strain of rat used in these studies (28 38 43 70 81, 83)

Nephritis produced in the rabbit with antirabbit kidney serums made in ducks has been described as closely resembling glomerulonephritis in man (13 31, 32 70) Hematuria and mild or moderate proteinuria accompanied by cylindruria are reported to appear after a latent period of about 5 to 8 days after the injection of nephrotoxic serums hypertension is a more frequent manifestation and hypercholesterolemia and edema are notably less prominent in nephrotoxic nephritis in the rabbit (13 70) than in the rat Chronic progressive lesions as well as azotemia and uremia also develop in rabbits (13 43 70)

In dogs, the intravenous injection of nephrotoxic serums produced disease resembling that seen in the rabbit The manifestations consisted of hematuria proteinuria cylindruria nitrogen retention and in some dogs mild hypertension and hypercholesterolemia (4 16) With rabbit antidog kidney serums no latent period was noted (4) using fowl antikidney serums there was a latent period before the onset of nephritis (16) As in the rabbit disease some dogs died in the acute phase of nephritis in some chronic nephritis developed and some appeared to recover completely In dogs too a form of heterologous protein nephritis similar to that described in the rabbit may develop (4)

IMMUNOLOGIC MANIFESTATIONS —The nature of the antigen-antibody reaction postulated in the pathogenesis of nephrotoxic nephritis has not been elucidated Inability to isolate and characterize the antigen or antigens involved has greatly hindered progress in resolving the differences in incidence and manifestations of nephrotoxic nephritis in various species after injection of antikidney serums of varying reactive capacity from fowls or rabbits It has been reported that renal disease resembling nephrosis is produced in rats by large doses of potent antirat kidney rabbit serum whereas nephritis like disease is said to follow the injection of small doses of this type of serum (13 43) Conflicting data have been presented by other workers who feel that nephrotoxic renal disease in rats generally resembles nephrosis and

of the interstitial tissues by mononuclear cells lymphocytes and poly morphonuclear cells These cells sometimes form aggregates around vessels and around glomeruli (4 27 81) Vascular and perivascular foci of cells have also been reported in other organs (4 81)

During the subacute and chronic stages 1 to 6 months after the onset of nephrotoxic disease the basement membrane becomes markedly thickened (13 27 39 70 81) Hyalinization of glomeruli accompanied by cellular proliferation and infiltration with the formation of adhesions between the glomerular tuft and the capsule are characteristic findings in some instances the adhesions partly or completely obliterate the glomerular capsular space (27 70 81) "Crescents" may occur earlier but are more prominent during this stage (39 70 81) Tubules are often dilated and may contain casts (27 39 70 81) Tubular cells may show hyaline or fatty degenerative changes and may be atrophied Interstitial infiltration continues to be present Finally glomeruli may become obliterated tubules markedly dilated or atrophied and a relative increase in interstitial tissue may be seen (27 70 81)

Many of the microscopic lesions described are not found in mild nephrotoxic disease (27 39 70) Lesions may consist of early changes in the basement membrane with minimal cellular proliferation If more extensive changes develop they may involve only a few glomeruli Restoration of essentially normal kidney cytoarchitecture has been noted after mild disease

It has not been possible to differentiate, by classic histopathologic studies nephrotoxic renal disease resembling glomerulonephritis from that which simulates the nephrotic syndrome (70) Renal biopsy has recently been developed and used in studies of the natural history of renal disease Electron microscopy has also been applied to this problem With these tools chronologic studies of human glomerulonephritis and nephrosis and comparative studies of nephrotoxic nephritis are being carried on in much greater histopathologic detail than formerly (53 54 88) Changes in glomerular structure consisting of a change of the cement substance between the basement membrane and the epithelial cells of glomerular capillaries changes in the density of foot processes of epithelial cells and thickening of the basement membrane have been reported in initial studies with the electron microscope of nephrotoxic disease in rats (54) These changes are said to parallel closely changes noted by means of the electron microscope in renal tissue obtained by biopsy from children with nephrosis (53

this form of nephrotoxic nephritis by a previous injection of duck serum and suppression of the reactions with roentgen rays are consistent with this theory (32) Histologic and histochemical observations of the proliferation and increased activity of mesenchymal cells in the rabbit kidney during the latent period following the injection of duck antikidney serum may indicate that antibody formed locally against bound duck antirabbit kidney antibody globulin is a reactant in nephrotoxic nephritis in the rabbit (80)

PATHOLOGY OF NEPHROTOXIC NEPHRITIS --The histology of the renal lesion in nephrotoxic nephritis like other features of this experimental disease, varies considerably depending on the size and the number of the doses of antikidney serum given (13 38) Small differences are noted in different strains of rats and in the lesion in the dog and rabbit as compared with the rat (13 38 70) Also it is apparent that there is a definite factor of individual variation among animals of the same strain or species (13 27 28 38 70) A composite chronologic picture of the histopathology of nephrotoxic renal disease cannot be compiled therefore The following description represents an abridged summary of the reports of the various investigations

Early in the acute stage, after the injection of antikidney serum swelling or thickening of the glomerular capillary basement membrane occurs accompanied by infiltration of the glomerular tuft by leukocytes (13 27 39 70) During the first few days of nephrotoxic nephritis swelling of endothelial and epithelial cells is observed in the glomerular tuft (27 39 70 81) and eosinophilic hyaline, and fibrin thrombi have been found in glomerular capillaries (27 70 81) The latter is a more prominent finding in severe nephrotoxic disease and is sometimes associated with partial or complete necrosis of glomerular tufts (27 70 81) Fibrin is seen in the capsular space and frequently in the dog and rabbit erythrocytes are present in Bowman s capsule (4 16 31 72) In kidneys of animals sacrificed or dead during the first or second week of the disease the capillary basement membrane is prominently thickened exudation into the glomerular capsule occurs and adhesions are found between the glomerular tuft and the capsule (13 27 70 81) Tubular changes are less pronounced than the glomerular lesions Swelling and granularity of some tubular cells accompanied by desquamation has been described (13 39 70 81) Eosinophilic hyaline casts are found in tubules (39 70) in the rabbit and dog red blood cell casts are occasionally seen (4 13 70) Tubular dilatation occurs (13 27 39 70) There is a varyingly intense infiltration

kidney antibody formation by collodion particle agglutination complement fixation and precipitation was unsuccessful (30)

From a review of the experimental data the conclusion that acute human glomerulonephritis following infection with nephritogenic streptococci has not been reproduced in animals seems reasonable It is also apparent that unequivocal experimental evidence supporting the hypothesis that autoantibodies to renal tissue components play a role in the pathogenesis of glomerulonephritis has not been obtained While many of the clinical and pathologic aspects of nephrotoxic and foreign protein nephritis in animals resemble corresponding features of acute nephritis in man, the fidelity and applicability of these experimental models as a means of investigating the pathogenesis of acute human glomerulonephritis appear to have one major limitation Although the assumption that human glomerulonephritis may be a hypersensitivity reaction is reasonable it would seem pertinent to intensify efforts to establish nephritogenic hypersensitivity in animals with group A streptococci rather than nephrotoxic sera or unrelated foreign proteins in view of the epidemiological evidence of the role of nephritogenic group A streptococci in this disease)

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88) Such changes have not been found in preliminary examinations of renal biopsy tissues obtained by biopsy from patients with glomerulonephritis (88)

Experimental Nephritis After Injection of Streptococci or Streptococci–Kidney Tissue Mixtures

Because of the epidemiologic evidence of the apparent role of certain types of group A streptococci in the etiology of glomerulonephritis a number of attempts have been made to produce nephritis in animals by infecting them with streptococci or by injection of streptococcal products In a few instances, signs indicative of a renal inflammatory reaction have been noted in experiments designed for other purposes (95), as well as in experiments planned specifically to produce nephritis (65) In general, these have been isolated observations and have not been reproduced by other investigators In a recent unconfirmed study hypertension microscopic hematuria and albuminuria were reported to have developed in rabbits given single or multiple subcutaneous injections of type 12 streptococci isolated from patients during an outbreak of glomerulonephritis (65) Similar results were described following the injection of filtrates of broth cultures of this strain of streptococcus Histopathologically however, the lesions were more compatible with minimal lower nephron nephrosis than with glomerulonephritis

Other attempts have been made to produce glomerulonephritis by stimulating experimental animals to produce autoantibodies which would react with their own kidney tissues Immunization with mixtures of streptococci and homologous kidney homogenates was the method used to produce renal autoantibodies In the most detailed study with this method (8) the development of nephritis was reported to occur in a high percentage of rats given streptococcus-kidney mixtures but rarely in similarly treated rabbits Antibody reacting with saline extracts of homologous kidney emulsions was demonstrated in the serum of the rats in which nephritis developed as well as in serum from rabbits in which nephritis did not develop (8) Antibody titrations were made with the sensitive but capricious collodion particle technic (7) The antibody titers were not quantitatively related to the presence or the severity of the nephritis A careful attempt to reproduce these findings employing some of the same strains of streptococci but with a different strain of rats was completely unsuccessful demonstration of anti

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Pathophysiology of Carcinoid Tumors

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In the short time since the recognition of the malignant carcinoid syndrome and the subsequent isolation of 5 hydroxytryptamine (5-HT serotonin) from the carcinoid tumor serotonin has become the current romance substance of medical research Experiments with this substance are being conducted at an intense and rapid pace that is only exceeded by speculations about its possible functions in the human body Currently it is often difficult to separate fact from fancy and any discussion bearing on the subject must be judged within such a frame of reference

Proper orientation requires a consideration of the enterochromaffin system the carcinoid tumor the malignant carcinoid syndrome the nature and pharmacology of 5 HT and related substances as well as the possible functions of 5 HT in the human body

ENTEROCHROMAFFIN (ARGENTAFFIN) CELLS

The cells believed to be responsible for the formation and secretion of 5 HT are known as the enterochromaffin cells Various other names have been used from time to time for the cells that constitute the enterochromaffin system such as gelbe zelle Kultschitzky cells the argentaffin cells Heidenhain (20) in 1870 was the first to recognize and describe them He isolated these cells from the stomachs of rabbits and dogs and demonstrated their chromaffin nature In man, normal enterochromaffin cells are present in the stomach particularly

phylogenesis Similar cells have been observed in the thymus of birds and the posterior salivary glands of Octopoda (10)

The function of these cells has long been the source of much speculation and has included such concepts as endocrine exocrine absorbative and excretory (48) In 1897 Kultschitzky postulated that they played a role in protein absorption Practically all of the hypotheses with the exception of the endocrine one are at present only of historic interest The recent claim that glucagon was a product of the argentaffin cell has received insufficient confirmation There is also inadequate evidence that any significant amount of histamine is either carried or secreted by them It is now recognized that these cells manufacture 5 HT from tryptophan and then secrete it into the circulation (11) Recent studies have also hinted at some relation between mast cells and 5-HT (3) In order to procure sufficient cell material for quantitative cell content studies it becomes necessary to study tumors derived from these cells Sjoerdsma and co workers in an exhibit at a meeting in 1957 showed the following quantities of 5 HT and histamine to be present in mast cell tumors and carcinoids

Tumor	*5 HT µg /Gm*	*Histamine µg /Gm*
Mast cell tumor (dog)	<0 2	315 160
Carcinoid tumor (man)	*360 570 800*	*34 20 0 8*

At present, therefore the only hormonal substance that has clearly been demonstrated within these cells in significant amounts is 5-HT

CARCINOID TUMOR

Tumors that today would undoubtedly be classified as carcinoids were observed over 100 years ago in the ileum and appendix, and were at that time considered to be atypical and undifferentiated carcinomas For years the tumor was called a "little" cell carcinoma In 1888 Lubarsch (26) reported 2 cases of *primaren Krebs des Ileum* The description given at that time unequivocally indicates that they were examples of carcinoid tumors Oberndorfer (35) 1907 suggested the term "carcinoid" for this form of neoplasm because of its relatively benign behavior and its carcinoma like histologic appearance The literature in the United States on the subject of these tumors was confused until 1925 when Forbus (14) redescribed and clarified some of their aspects The German pathologists were in agreement that

at the base of the cardiac and pyloric glands in the villi and crypts of the small and large intestines and in the appendix the mucosa of the gallbladder and in the pancreatic ducts They are believed to form a diffuse endocrine gland Gosset and Masson (18) anticipated the recent discoveries by ascribing a neuroendocrine function to these cells over 40 years ago Erspamer (10) while studying the nature of the granules in these cells isolated a substance from gastrointestinal mucosa which caused contraction of smooth muscle and named it enteramine Rapport and co workers (42) independently discovered a substance in serum while searching for a vasoconstrictive factor and named it serotonin 3 9 With the identification of 5 HT it was realized that enteramine and serotonin were the same substance (10)

The body of the enterochromaffin cell usually adheres closely to the basement membrane and bulges into its outer surface A constricted continuation extends from the cell toward the lumen Its nucleus is large and spherical, while the oval nuclei of the surrounding epithelial cells project beyond it The cytoplasm at the base of the cell is filled with granules which so intrigued Erspamer and associates They stain easily with eosin acquire a brownish yellow color on exposure to chromates and show the classic argentaffin reaction when treated with ammoniacal silver nitrate The argentaffin reaction has at times been confused with silver impregnation in which reducing agents are used, but the latter is correctly called an argyrophilic reaction In the true argentaffin reaction the silver salts are reduced by the tissue component stained However this will occur only if the tissue is fixed in formalin if alcohol is used the ability of the tissue to reduce ammoniacal silver salts disappears The authentic argentaffin reaction not the argyrophilic one is characteristic of the enterochromaffin cell and the cells of the carcinoid tumor Many investigators have studied the detailed histochemistry which indicates that the vacuoles in the cells contain a mixture of neutral fats cholesterol esters and a little lecithin various opinions about the granules implicate a pterin a carbohydrate a derivative of resorcinol and a freely conjugated β carboline derivative (43)

Some claim that the cells arise from entoderm while others notably Lewis and Geschickter (25) believed their origin to be from neuroectoderm Among the vertebrates the Cyclostomata lack typical enterochromaffin cells but they do have cells referred to as pre enterochromaffin cells These cells may have lost their 5 HT content during

be the favored site Carcinoid tumors of the appendix are generally found in young individuals because in this region the tumor more often produces symptoms and almost any significant lower abdominal discomfort in a young individual often results in an appendectomy Without adequate explanation it has been noted that carcinoids of the appendix occur more often in females than in males whereas in the other sites of origin there is no sex predilection.

In the appendix the carcinoid is most often located at or near the tip and often appears as a submucosal intramural yellow nodule The overlying epithelium is intact In the intestine, too, the tumor is intra mural, and often consists of a small submucosal nodule At times it

TABLE 2 —Sites of Origin of 509 Carcinoids as Reported in Literature

Site	Carcinoid	
	Number	Per Cent
Appendix	341	67
Ileum	77	15
Jejunum	7	2
Small intestine, undetermined	33	7
Colon	18	4
Rectum	13	3
Stomach	9	2
Duodenum	5	1
Gallbladder	1	0 2
Unknown origin	5	1

may be as large as 15 cm in diameter Polypoid annular, and constricting patterns of growth may also occur Sometimes as the tumor enlarges central umbilication with ulceration of the overlying mucosa is seen

It is now established that the carcinoid tumor arises from the cells of the enterochromaffin system This was first proposed in 1910 according to Ritchie (43) but it was Gosset and Masson (18) who supplied the necessary supporting data and who evolved a theory of origin for these tumors Because rectal carcinoids do not exhibit the characteristic granules seen in the cells of the tumor in other sites Stout (56) has suggested that rectal carcinoids may originate from the pre enterochrome type of cell observed in other animal species This is significant because malignant rectal carcinoids even with extensive hepatic metastasis have to date not been associated with the malignant carcinoid syndrome

The tumor typically consists of well defined solid clumps or strands

these tumors arose from the enterochromaffin cells of the gastrointestinal tract For a long time these tumors were regarded as medical curios without much clinical importance Their malignant nature was not appreciated nor was there any suspicion of any clinical syndrome secondary to chemical secretions from these tumors

The carcinoid tumor or argentaffinoma may occur at any point within the gastrointestinal tract from the cardiac end of the stomach to the anus It is frequently observed in the appendix and ileum occasionally in a Meckel s diverticulum or the gallbladder and rarely in teratomas of the ovaries and testes Such cases as are reported as primary carcinoid of the mesentery and pancreas must be regarded

TABLE 1 —APPENDICEAL AND NONAPPENDICEAL SITES OF CARCINOID IN SURGICAL AND AUTOPSY MATERIAL AS REPORTED IN LITERATURE

Site	Surgical Material		Autopsy Material	
	Number	Per Cent	Number	Per Cent
Appendiceal	181	92	8	11
Nonappendiceal	15	8	65	86
Undetermined	0		2	3
Total	196		75	

as unproved because there is insufficient evidence in the records that primary tumors elsewhere such as in the ileum had been definitely excluded

A form of bronchial tumor designated as the carcinoid type of bronchial adenoma is unrelated in its histogenesis to the argentaffinoma of the gastrointestinal tract The continued use of this term in reference to a particular type of bronchial adenoma is confusing and is better discontinued Recently however a carcinoid like tumor has been described in the bronchial tree associated with the syndrome (33)

Tables 1 and 2 list the sites of origin of carcinoid tumors as reported in the literature The relative incidence of occurrence in the appendix as compared with the intestine will depend on the source of the material analyzed If the series consists entirely of surgically removed specimens the vast majority will be in the appendix If the study is based on autopsy material the intestine and especially the ileum will

noids 33 per cent were found to be noninvasive 27.3 per cent had invaded muscle 23.3 per cent involved the regional lymph nodes and 16.7 per cent had metastases in distant organs a total of 67 per cent revealed either local invasion or metastases (43)

Although local invasion of muscular coats is often noted in appendiceal carcinoids only a few authenticated cases with distant metastases have been reported This low incidence of metastasis from appendiceal carcinoids may be explained in part by the relatively slow growth of the tumor combined with frequent early removal of the appendix In the appendix, as well as in other sites local infiltration is often accompanied by hyperplasia of adjacent muscular tissue Distant metastases are found commonly in the liver, but almost any organ may become involved

The microscopic appearance is of little aid in the prognosis of the rate of growth or the development of metastases The cellular pattern is usually uniform, but variations in cell type and bizarre cells may be present in both the "benign" and malignant variants Atypical cells are at times more numerous in the more invasive tumors (37)

Survival in the presence of liver metastases has been as long as 25 years Carcinoid tumors occur at all ages and there is 1 reported case in a 10 day old infant, Those with metastases are almost always seen after the age of 45. This is further indication of the sluggish growth and slow tendency to spread (34 40)

The current tendency is to consider all carcinoids potentially malignant

Secretion

Evidence that carcinoid tumors secrete an active physiologic or pharmacologic substance has been known since 1924, when extracts from an appendiceal carcinoid exhibited properties similar to epinephrine (7) Since then, others have extracted pressor materials from these tumors (13 46) A most significant contribution was made by Lembeck (24) who isolated considerable amounts of 5-HT from carcinoid metastases and from a "benign" appendiceal carcinoid. This has since been repeated by others (49) The 5-HT so obtained is identical to the enteramine isolated by Erspamer and the serotonin of Page (36) and Rapport *et al* (42)

5-HT or serotonin as it is most commonly known, belongs to the family of indolealkylamines and has the formula shown in Figure 1.

The 5-HT content varies from one tumor to another and in different

of rather small closely packed polyhedral cells There may be scattered areas of small glandular lumens or distinct acini The more actively growing tumors show varying degrees of anaplasia Mitoses are usually rare and it is practically impossible on histologic evidence alone, to determine their benign or malignant potentialities Variations in cell types may have functional significance The special staining characteristics reflect those found in the cell of origin (enterochromaffin cell) Many of the stains depend on artifacts associated with formalin fixation The tumor itself gives *(1)* a positive chromaffin reaction, *(2)* stains with iron hematoxylin and with acid and alkaline diazonium salts *(3)* is argentaffin with silver nitrate *(4)* shows yellow fluorescence with ultraviolet light and *(5)* on frozen section reveals droplets that stain partly with scarlet red Sudan IV and osmic acid Doubly refractile crystals are observed under polarized light (44 45)

The frequency with which the tumor arises in the same individual from multiple sites raises the question whether it may be developmental in origin This view was among the earliest held concerning the pathogenesis of these tumors Such terms as "*hamartomartige Bildungen*" and mucous membrane naevi have been used in this connection (50) In support of this are reports of association with minor congenital cardiovascular abnormalities (4 cusped pulmonary valves Chiari s networks dextrocardia) Meckel s diverticulum cleft palate and cystic teratoma (12 53) In fact these tumors may arise in cystic teratomas of the ovaries or testes and are second in frequency only to squamous cell carcinoma in tumors growing from dermoid cysts of the ovary (12) Some have reported a significant association with other malignant tumors (37)

MALIGNANCY

The malignant potential of the carcinoid tumor was not fully recognized until the past decade although such a tumor with metastases was described in 1890 by Ransom (41) Despite the fact that over 400 cases with metastases have been reported as late as 1939 Cohen and associates (6) claimed that the tumor did not *metastasize*

Primary carcinoid of the ileum metastasizes more often than those arising from other sites The tumor at first grows intramurally beneath the mucosa It may spread into the adjacent muscular coats then into the regional lymph nodes into the blood vessels and finally to the liver and other organs In one study, on 146 extraappendiceal carci

secondary to hyperserotonemia Many carcinoids are asymptomatic and are only discovered as incidental findings at autopsy; others may show recurrent or chronic partial intestinal obstruction The incidence of obstructive manifestations varies between 25 and 50 per cent of all small bowel carcinoids (23 37) Intermittent diarrhea, pain and loss of weight complete the picture The tumor is seldom palpable through the abdominal wall The diarrhea is often bloodless because the growth is intramural and usually without ulceration of the over lying mucosa. Intussusception is an occasional complication

In general, the clinical features which depend on the presence of the mass itself or at sites of metastases are no different than those due to masses of the same size and location but of a different histogenesis

Rectal carcinoids may be locally extensive and infiltrate into the surrounding perirectal tissue Their behavior simulates that of rectal carcinoma, from which they are as a rule clinically indistinguishable Bleeding is more common at this site A biopsy specimen of such a lesion may be mistaken for carcinoma or else reveal no tumor cells because of the deep submucosal location of the tumor It has already been mentioned that rectal carcinoids may not display the tinctorial characteristics of an argentaffinoma Since the affinity of the cellular granules for certain stains is related to the presence of 5-HT it is not to be expected that rectal carcinoids even with extensive hepatic metastases will be functional in the sense of producing the "ma lignant carcinoid syndrome"

Anemia is a late manifestation of the localized form of intestinal carcinoid, and in this respect the carcinoid differs from other malig nant neoplasms of the intestine

Malignant Carcinoid Syndrome

The clinical picture of the malignant carcinoid with metastases in which there is significant hyperserotonemia has been extensively de scribed in the last few years (33 49 58) For the pharmacology and distribution of serotonin, *see* page 221

The specific signs and symptoms are believed to be related to ex cessive production and secretion of 5-HT Although a "benign" car cinoid may form and secrete increased amounts of serotonin, the clinical syndrome has so far only been described in association with metastatic malignant carcinoid in which larger numbers of functioning cells are present Recent study of a "benign" appendiceal carcinoid

parts of the same tumor In one instance a metastatic nodule contained 360 μg per gram while the primary tumor contained 61 μg Most tumors so far analyzed have yielded over 200 μg per gram (49)

In the normal individual, 5 HT is usually detected in the blood, spleen gastrointestinal tract and central nervous system Elevated

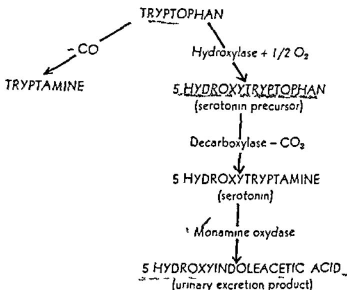


Fig 1

serum levels of 5 HT have been found in the presence of the tumor Urinary 5 HT and 5 hydroxyindoleacetic acid i e the breakdown product of 5 HT are increased in individuals with metastatic carcinoid tumors This finding is used as a diagnostic aid for the clinical detection of carcinoid A simple test, based on the development of a specific purple color reaction for 5 hydroxyindoles when 1 nitroso 2 naphthol and nitrous acid are added has been devised by modifying and simplifying the method for quantitative assay of 5 hydroxyindoleacetic acid (60)

Local Effect of Carcinoid of Ileum

The clinical picture of carcinoid tumors may be divided into two parts—that associated with the local mechanical and space-occupying effects in the intestine, and that associated with the systemic effects

the malignant carcinoid syndrome depends only slightly on local mechanical irritation In all probability it is related to the stimulation of smooth muscle by 5-HT and to the associated localized intestinal muscular hyperplasia The diarrhea is often an early and persistent manifestation The stools are watery and may number as many as 30 a day

The changes in the skin especially of the face include teleangiectasia and dilatation of the small vessels This imparts a plethoric appearance to the individual, although there is no increase in the total number of red blood cells In most cases there are episodic attacks of flushing associated with extreme cyanosis At times they are combined with such vasomotor manifestations as sweating and hot sensations The hemodynamic changes during the flushing period have been studied in detail (57) During the early stage of the flush the cardiac sounds murmurs and contractions and the ballistocardiographic waves are diminished As cutaneous vasodilatation is completed all of the above increase considerably This is usually accompanied by tachycardia rise in blood pressure hyperpnea and tachypnea With the appearance of cyanosis the findings revert to those seen at the onset of the flushing episode These manifestations are not always constant The episodes may be fleeting or last many hours and may be evoked by a variety of stress situations

In some there are recurrent episodes of cyanosis together with respiratory symptoms akin to bronchial asthma the patient is dyspneic wheezes and has an expiratory stridor The blood pressure remains relatively normal during the attack

At a meeting in 1957 Weissbach discussed the implications of the presence of 5-HT in the lung and the anaphylactic reaction in general The enzymes which build and destroy 5-HT are also present in relatively high concentration in the lung in normal animals of various species It has been shown that the addition of purified antigen and antibody to normal rabbit platelets releases 5-HT and histamine *Guinea pig lung is low in histamine and high in 5 HT* antihistamines are practically useless in the guinea pig while lysergic acid diethylamide (LSD) which has an antiserotonin action may protect the animal against anaphylaxis 5 HT plasma levels may be increased tenfold in experimentally induced anaphylaxis in rabbits Further studies on the quantitative determination of the metabolites of 5-HT in the urine of patients with various allergies may yield valuable data and insight into the general problem of allergy

revealed a 5 HT content 1 000 times greater than that of normal tissue, and an enzymic ability to synthesize 5 HT from its precursor substance 35 times that of normal human appendiceal tissue

The basic findings of this syndrome consist of chronic diarrhea flushes and cyanosis respiratory distress and right sided cardiac disease. Thorson *et al* (58) were the first to suggest that these protean manifestations might be secondary to the presence of excessive amounts of 5 HT Since 5 HT acts on the smooth muscle of blood vessels bronchi and intestine he was justified in implicating this substance Sjoerdsma *et al* (49) in extensive metabolic studies on tryptophan intake and utilization in normal individuals and patients with this syndrome demonstrated a marked depletion of the body reserves of tryptophan as a result of the synthesis of large amounts of 5-HT

5 HT is formed as a result of a ring oxidation and decarboxylation of tryptophan In the formation of 5 hydroxytryptophan by means of a hydroxylase 60 per cent of the tryptophan in the body may be utilized As a result of this there may be inadequate niacin and other protein synthesis In this condition a nutritional deficiency may develop indeed a pellagra like picture has been reported (31) The concept that tryptophan is a precursor of 5 HT and of 5 hydroxyindolacetic acid was supported in man through tracer experiments with C^{14} labeled tryptophan (*see* Fig 1)

Palpation and massage of the tumor have precipitated characteristic flushes and respiratory distress In the same individual histamine injections apparently also produced some aspects of the clinical syndrome It is postulated that histamine may stimulate the carcinoid to release its secretion just as it stimulates a pheochromocytoma to release norepinephrine (34)

Thus both excessive secretion of 5 HT and tryptophan depletion must be considered in any attempt to provide a rational basis for the entire clinical picture and the anatomic changes in the cardiovascular system

Ordinarily the blood concentration of 5 HT ranges from 0 1 to 0 4 µg per milliliter, and the 24 hour urinary output of 5 hydroxyindole acetic acid normally ranges between 3 and 8 mg But in the carcinoid syndrome the serum levels of 5 HT have been as high as 4 µg per milliliter and the urinary output of 5 hydroxyindolacetic acid as high as 330 mg

The diarrhea in patients with the "benign" carcinoid as well as with

coagulum or a nonbacterial type of vegetation has been associated with the lesion (30 53) However these may be nonspecific secondary changes and not necessarily pathogenetically related to the lesion The chordae tendinae may be surrounded by a uniformly cellular fibrous thickening At the base of the valves a few lymphocytes and macrophages may be noted MacDonald and Robbins (28) have observed increased numbers of mast cells and have speculated on their relation to the development of the valve changes The lesion appears to be distinct and can generally be distinguished from congenital valve changes fibroelastosis rheumatic valvulitis collagen disease and thrombotic or bacterial endocarditis

The tricuspid and pulmonic valves appear to be involved with equal frequency In 1 series the mitral valve was reported to be involved in 12 of 35 cases and the aortic valve in 5 cases (it is not clear whether coincidental rheumatic involvement of the mitral valve was excluded in some of these cases) the pulmonary artery was involved in at least 3 cases and the endocardium of the right auricle revealed fibrous thickening in about one third of the cases (51)

The occurrence of hypertrophy and dilatation of the right auricle and ventricle with or without congestive failure depends on the extent and type of valve deformities The other clinical features of a functioning malignant carcinoid tumor may be present without any evidence at autopsy of anatomic cardiovascular changes In a review of 26 carcinoids–benign and malignant–found at autopsy 10 of the cases revealed various degrees of right sided endocardial or valvular changes some of them in the absence of metastases (53) On the other hand, it has been claimed that the specifically characteristic cardiac lesion occurs only in the presence of hepatic metastases It has also been maintained that the characteristic left sided cardiac lesion can occur only in the presence of a patent foramen ovale This is not borne out by our personal experience

The pathogenesis of the cardiovascular changes is still obscure Various mechanisms have been proposed to explain the predominantly right sided endocardial involvement. Gobel and co workers (17) offer at least a partial explanation after collecting blood simultaneously from the brachial and the pulmonary arteries they found the plasma 5-HT content of the brachial artery to be much lower than that of the pulmonary artery they estimated that about two thirds of the unbound 5-HT was removed in the passage through the lungs This finding however remains unconfirmed They attributed this to the high pulmonary con

Edema of the legs pleural effusion and arthritis have also been observed The finger joints were the main site of involvement However all of the cases with arthritis are in that age group where degenerative joint changes are frequent (33 43)

It is difficult to evaluate evidence of mental disturbances because most of the patients were seriously ill However in the clinical records of these patients such phrases as uncooperative unreliable contradictory and very nervous are not uncommon The main anatomic manifestation of this syndrome with its resultant symptomatology is localized in the cardiovascular system In the advanced form it consists primarily of fibrostenotic lesions of the pulmonic and tricuspid valves

Cardiovascular Changes

At least 70 acceptable cases of valvular right sided lesions have been reported in association with malignant carcinoid tumors with hepatic metastases (27 33 43) The gross and microscopic changes in the valves endocardium and pulmonary arteries in this syndrome have recently been described in detail (28 30 51)

Lesions are found chiefly in the pulmonic and tricuspid valves The auricular and ventricular endocardium of the right cardiac chambers *as well as the intima of the major pulmonary arteries may also* be affected The question whether specific lesions of the left cardiac chambers occur in the absence of a patent foramen ovale is still being debated

The lesion consists of a plaque of moderately cellular tissue intimately adherent to the surfaces of the valves or the other involved sites e g pulmonary artery intima The valve itself appears to be intact, although adhesions between the cusps or leaflets may form eventually and finally lead to marked valvular distortion The process may extend to the adjacent endocardium and the chordae tendinae The plaques are made up of a relatively acellular ground substance containing fibrous tissue cells most of which are spindle shaped The ground substance is homogeneous and basophilic and contains delicate fibers elastic fibers are few or absent entirely There is no lipid within the lesion and special stains fail to reveal surface or incorporated fibrin Periodic acid stain is only slightly positive Foci of distended capillaries are present in the subendothelial thickening superimposed upon a *relatively* intact endocardium (28) In a few cases a fibrin

the more rapid growth rate of the carcinoid might be secondary to the valvular lesion, which they regarded as congenital

At present, it is generally held that excessive amounts of 5 HT circulating through the right cardiac chamber and pulmonary circulation are pathogenetically related to the endocardial sclerosis but the mechanism of its action remains obscure

PHARMACOLOGY AND DISTRIBUTION OF SEROTONIN

At present, it is believed that 5 HT is elaborated by the enterochromaffin system It is found in the cells of this system in the blood, especially within the platelets in the spleen and in some cerebral and peripheral nervous structures (10) Serotonin may be identified quantitatively in tissue by specially designed spectrofluorometric techniques Chemical isolation color reactions paper chromatography and pharmacologic bioassay methods have all been used. The spectrofluorometric method is especially useful for central nervous system tissues (61)

The substance is released from the cells in the gastrointestinal tract into the blood where most of it is taken up by the platelets Tryptophan is probably the parent substance of most of the naturally occurring indole derivatives Enzymic destruction of 5-HT into an indol acetic acid is accomplished by monamine oxidase The monamine oxidases are present in greatest concentration in the lung and brain (10) It is claimed that there is a threshold rate for every animal species which limits the capacity of the blood thrombocytes to take up 5-HT Little is understood about the conditions which regulate possible physiologic release by intact thrombocytes Cellular disintegration is probably not essential to the liberation of 5 HT from the platelets (10)

The estimated normal serum level of 5-HT is 0.1 μg per milliliter The metabolic rate of endogenous 5-HT as determined by the urinary output of 5-hydroxyindolacetic acid is sufficiently intense not to support the theory of disintegrating platelets as the only source of plasma 5-HT Most of the 5-HT in the circulating plasma can be attacked by the monamine oxidases particularly as the blood circulates through the lung (10)

The identity of synthetic serotonin and the naturally occurring material has been demonstrated by numerous pharmacologic reactions Only scant data are available on the toxicity of 5-HT The intravenous

tent of monoamine oxidase, the enzyme which converts the CH NH₂ group of 5 HT to CO H and thereby produces hydroxyindoleacetic acid The latter substance is a urinary excretion product and is pharmacologically inactive They found monoamine oxidase in other organs as well

It is also possible that the liver has a role in 5 HT metabolism. Extensive amounts of 5 HT, secreted by metastatic carcinoid nodules in the liver, enter the right side of the heart before passing through the lungs so that presumably large amounts of 5 HT may then act on the endocardium and produce the observed changes On the other hand the changes need not be the result of a specific 5 HT effect on the endocardium but rather of hemodynamic disturbances in the heart and pulmonary circulation In animals 5 HT has produced a marked rise in pulmonary blood pressure This has been confirmed by means of pulmonary arterial catheter studies in a few patients with the malignant carcinoid syndrome (30) It is therefore possible that the anatomic lesion may be secondary to the mechanical stress of disturbed hemodynamics The latter concept of course cannot explain left sided cardiac lesions in the absence of a patent foramen ovale Another point of view holds the tryptophan deficiency to be at least a contributing factor in the development of the cardiac lesions It is well known that in the South African Bantu who subsists on a diet of mealy meal (corn meal) and suffers from essential amino acid deficiencies endomyocardial fibrosis may develop Whether the excess tryptophan utilization with depletion of its reserves can effect a similar change is problematic

There are no reports as yet of the experimental production of the cardiac lesion by long term administration of 5 HT Rabbits given massive daily doses for 3 months failed to show any comparable cardiovascular lesion (53) Prolonged administration of 5 HT might possibly result in valvular changes but this would not answer the question whether the substance acts directly on the endocardium or by way of changed hemodynamics Because 5-HT is carried by the platelets thrombosis has been suggested as a possible factor in the pathogenesis of the sclerotic plaques However evidence so far does not support this thesis Furthermore platelet adhesiveness does not seem to be increased in this condition In rats subcutaneously administered 5-HT produces a localized edema rich in protein whether this is the initial change in the valve remains to be demonstrated Hedinger and Gloor s (19) thesis a view not generally accepted is that

mines may be classified by their effects on (1) systemic blood pressure (2) special vascular areas (pulmonary vessels) (3) isolated artery strips (4) the heart (5) extravascular smooth vessels such as urinary bladder and smooth muscle (6) respiration (7) impulse transmission in ganglions (8) central nervous system (9) development of cutaneous pain (10) certain metabolic effects (11) adrenal medulla (12) hista mine releasing activity and (13) participation in hemostatic mech anisms

Serotonin seems to form complexes readily many of which are quite stable This may be of significance in its biologic activity

SEROTONIN AND CEREBRAL FUNCTION

Twarog and Page (59) were among the first to note that 5-HT is present in cerebral tissue they like other investigators at that time used bioassay methods Development of a specific spectrophotometric technic has now made possible rapid advances in knowledge of the metabolism and the pharmacologic properties of 5-HT in cerebral tissue Its high concentration in certain areas of the brain and the marked effects of indole compounds on the central nervous system suggest that it may play an important part in cerebral function The largest amounts of 5-HT are found in the so-called primitive cerebral areas such as the midbrain and especially the hypothalamus the cor tex and cerebellum contain much smaller amounts Decarboxylase and monoamine oxidase the enzymes necessary for 5-HT synthesis and destruction respectively are also present in the brain Significantly monoamine oxidase although found throughout the brain, is present in highest concentration near the hypothalamus The distribution of decarboxylase which converts 5-hydroxytryptophan to 5-HT also parallels that of 5-HT (15 61)

It is not yet known whether the brain can synthesize 5-HT from its basic precursor tryptophan In animals the cerebral 5 HT content has been raised by administration of large doses of 5-hydroxytryptophan Since 5-HT does not readily penetrate the blood brain barrier but 5-hydroxytryptophan does (61) administration of the latter substance has been proposed as possibly a practical method of increasing the cerebral 5-HT content in man Reactions to excessive amounts might be controlled by the use of antimetabolites

In the experimental animal it is possible to obtain 5-HT levels

LMD_{50} is 160 mg per kilogram for mice and 30 mg per kilogram for rats Dogs and human beings tolerate relatively large amounts administered by rapid intravenous infusion—0 01 to 0 2 mg per kilogram However disagreeable symptoms appear with the larger dosages To date experimental duplication of dosages quantitatively similar to those occurring with an actively secreting metastatic carcinoid has not been feasible in man (10)

The variable behavior as well as variation in dose response in different species renders any analogies to man based on animal studies uncertain

Among the protean pharmacologic findings action of 5 HT on smooth muscle is the most readily reproducible and was the first to be discovered In animals it may raise the blood pressure and cause uterine and intestinal contraction The renal blood vessels of the dog are highly sensitive to 5 HT One pronounced serotonin effect is evacuation of the bowels, with increased peristaltic sounds, and increased tone of the urinary bladder

Its effects on the respiratory system differ according to the animal tested In some there is immediate respiratory standstill in rabbits a marked increase in right ventricular pressure occurs in guinea pigs dyspnea develops and is followed by convulsions In man the response to intravenously administered 5 HT resembles somewhat the response in the dog exaggerated respiratory movements, cough and tachycardia with urination in addition the symptoms in man range from none at all to a feeling of tightness of the chest, generalized tingling and prickling itchy nostrils, difficulty in breathing, desire to empty the bladder associated with pain in the stomach as well as the bladder weakness nausea desire to sneeze generalized numbness and burning of the throat mouth and hands

The response of patients with hypertension varies It is noteworthy that the 5 HT serum levels in hypertensive patients is no higher than that found in normotensive individuals and in some is even lower This finding certainly does not support the view that 5 HT maintains increased vascular tone in individuals with hypertension (8 21) In analyzing the physiologic action of 5-HT only those reactions can be considered physiologic which are provoked in an intact animal by doses lower than the total 5 HT content of the organism All other reactions must be considered pharmacologic

Various physiologic or pharmacologic actions of the indolealkyla

mines may be classified by their effects on (1) systemic blood pressure (2) special vascular areas (pulmonary vessels) (3) isolated artery strips (4) the heart (5) extravascular smooth vessels such as urinary bladder and smooth muscle (6) respiration (7) impulse transmission in ganglions (8) central nervous system (9) development of cutaneous pain (10) certain metabolic effects (11) adrenal medulla (12) hista mine releasing activity and (13) participation in hemostatic mech anisms

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In the experimental animal, it is possible to obtain 5 HT levels

that are 20 times the normal level animals with these high levels become excited and reveal tremors The simultaneous administration of reserpine and iproniazid (monoamine oxidase inhibitor) produces the same picture in animals The effects are attributed in both instances to an excessive amount of unbound 5 HT Administration of 5-hydroxytryptophan to animals pretreated with iproniazid raises the 5-HT levels but not that of its precursor The cerebral manifestations of the animals therefore cannot be ascribed to an excess of 5 hydroxytryptophan It is also interesting that the cerebral 5 HT content of pyridoxine-deficient chickens is only 30 per cent of normal and the level in the other tissues is even more drastically reduced Perhaps some of the neurologic disturbances in this type of deficiency may be due, in part to a change in 5 HT content However oversimplification of this problem is hazardous because the same chemical and enzymic systems are involved in the formation of epinephrine and histamine (61)

In 1943, Hoffman (22) while working in his laboratory, noticed peculiar sensations of vertigo and restlessness he was in a dreamlike state and appeared drunk his imagination became exaggerated and when he closed his eyes he saw fantastic and vividly colored pictures this state lasted for 2 hours As it turned out, he had been working with *d* lysergic acid diethylamide (LSD) and had accidentally inhaled a small quantity It is now known that tiny doses of LSD or of its bromine derivative block the action of 5-HT Other antagonists of 5-HT action on uterine smooth muscle exert the same effect on the brain Among these naturally occurring alkaloids that are antimetabolites of 5 HT are the harmala alkaloids (harmine and yohimbine) and the ergot alkaloids All of these are structurally related and contain the indole nucleus Reserpine is an analogue of yohimbine (16 47) The antagonism of chemical substances of a similar molecular structure has been likened to that of a lock and key The right key is needed to produce an effect A similar key can fit into the lock and block the keyhole even though it cannot open the door

The fact that in man LSD produces schizophrenia like symptoms such as change in perception changes in body image and time sense impairment of integration and fragmentation of mental processes is significant Only a minute amount of drug is needed to induce these phenomena comparable to effective doses of the most potent hormones and vitamins suggestive that LSD has a highly selective action on the central nervous system It is now believed that the mechanism of LSD

action is its antagonism to 5 HT (5 9) Psychic effects may be observed after administration of only 1 μg LSD per kilogram body weight, but Waldenstrom (62) administered up to 1 500 μg of LSD to a patient with malignant carcinoid without the occurrence of any psychic changes In a similar situation Snow and co workers (52) gave 7.5 mg of LSD daily for several weeks without observing any mental changes the effect on the symptoms of the malignant carcinoid syndrome however were insignificant It is therefore premature to attribute the mental aberrations produced by LSD entirely to its antimetabolic action on 5 HT At a meeting of the American Psychiatric Association 2 years ago (1) it was concluded "The psychotic state produced by lysergic acid diethylamide was felt not to be identical with that found in schizophrenia but there were sufficient similarities to suggest a biochemical factor is involved in schizophrenia " Changes in 5 HT metabolism may eventually prove to be one of the keystones in the possible biochemical factors which play a role in the causation of schizophrenia At this stage however the administration of 5-HT to schizophrenic patients has failed to produce any beneficial effects (32)

There is considerable evidence that the primary pharmacologic effect of reserpine is to impair 5 HT binding sites thereby releasing 5-HT from the enterochromaffin system and preventing its storage Reserpine disappears from the brain rapidly in contrast to its persistent effects For these and other reasons it is believed that the reserpine effects are mediated through the release of 5-HT (38) The observation that an LSD induced deficiency of free 5 HT and an excess of free 5 HT may result in similar central nervous system effects is provocative It is postulated that 5-HT may act as the chemical transmitter of nerve impulses to the centers of the parasympathetic division (5)

Attempts to construct a scheme showing the relations of the possible balances and imbalances between cholinergic excitation and adrenergic inhibition as these are related to 5-HT metabolism are still premature but the answer to many unsolved problems of mental derangement may lie in this scheme

Evidence for a key role of 5-HT as a chemical mediator in nerve impulse transmission may be noted in a wide variety of animals 5 HT is found in the venom of the wasp and in the tentacles of stinging nematocysts The function of 5-HT at these sites may be to aid in defense or help in the capture or relaxation of prey In many crustaceans 5-HT or a closely related substance has been found in leg nerves The decapod

crustacean s heart is neurogenic and an indolamine substance (either 5 HT or one quite similar) acts as a cardiac regulator in these invertebrates (63)

Serotonin and Certain Blood Dyscrasias

Experimental studies on hemostasis in animals reveals that 5 HT released from the platelet plug partly accounts for the vasoconstriction which helps to prevent bleeding Certain reports have indicated the existence of a 5 HT deficiency in the serum of patients with thrombocytosis and thrombopenia (64) Subnormal concentrations have also been noted in a few patients with thrombocytopathia and pseudohemophilia however patients with the severest hemorrhages in these conditions have had normal serum 5 HT levels (4) Thus, in all these conditions the major cause of the bleeding cannot be ascribed to a 5-HT deficiency although in some it may through interference with vasoconstriction contribute toward the bleeding tendency

Serotonin and Renal Function

Erspamer (10) believes that a basic physiologic action of 5 HT is the regulation of renal circulation and function An antidiuretic action of 5 HT has been demonstrated in man dog rat guinea pig and rabbit The mechanism of this action in the various species is not entirely similar or clear In the rat it appears to be mediated through a reduction in the glomerular filtration rate With excessively large doses of 5-HT the afferent glomerular arterioles becomes markedly constricted

Some investigators have disagreed with the concept of both of the above mechanisms and claim that the antidiuretic action is mediated through the posterior pituitary (55) In dogs it has been demonstrated that much of the antidiuretic action may be due to increased tubular reabsorption of water However chronic intravenous infusion with physiologic doses in unanesthetized dogs produced a definite antidiuretic effect at a threshold dose of 10 μg per kilogram per minute and became more pronounced until a dose of 20 μg was reached thereafter further increases in dosage were without effect. Analysis of the data in relation to urine clearance supported the concept of an increase in the rate of tubular reabsorption of water The antidiuretic action occurs in the absence of any change in systemic blood pressure

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Some Aspects of Disordered Renal Tubular Function

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ALTHOUGH IT IS now customary to speak of "disorders of renal tubular function" or of "renal tubular dysfunction," these terms are often applied after only the most casual attempt to prove that an observed renal abnormality originates in disturbance of specific tubular mechanisms There are many obstacles in the way of establishing such proof not the least of which is our almost total ignorance of the intimate cellular mechanisms that mediate the transport of substances across the renal tubular wall. It is however possible to define approximately the magnitude of the reabsorptive processes effected by the tubules Collectively in the normal individual they return to the blood 99 per cent of the water and most of the mass of solutes contained in the 180 litres of ultrafiltrate formed daily by the glomeruli The renal tubule can thus be regarded primarily as an organ of conservation and, through the selective reabsorption of various substances and the secretion of hydrion (166) and probably of potassium (23) it plays a major role in homeostasis.

In clinical practice the appearance in the urine of substances that normally are completely reabsorbed, or the development of some obvious disturbance of renal homeostatic function, is too readily taken to indicate tubular malfunction For example since a normal individual reabsorbs all the glucose filtered by the glomeruli in the course of the day the occurrence of glycosuria in the presence of normal plasma levels of glucose is commonly ascribed to impaired transport of glucose by the tubular cells and, since a minor deficiency of sodium

But in other congenital disorders, such as the Lignac Fanconi syndrome the innate defect may be complicated by progressive destructive changes in the kidney it may then prove as difficult to recognise a true tubular defect as it is in any other form of destructive renal disease Moreover when renal tubular involvement is a secondary consequence rather than a primary manifestation of a genetic disorder the extent of tubular malfunction may vary from patient to patient. This is best seen in Wilson's disease Such varying degrees of secondary "toxic" effect must be distinguished from the qualitative or quantitative differences that sometimes may occur between homozygous and heterozygous individuals.

An attempt to survey all the conditions thought to involve disordered tubular function would entail consideration of almost all aspects of renal disease and necessitate many incursions into pure physiology Consequently the following discussion is selective choice of topics has been determined partly by personal interests and experience and partly by their relevance to the clinical practice of internal medicine Several important subjects have been omitted but renal glycosuria (30 123 173) and the tubular lesion of "pseudohypoparathyroidism" (112) have recently been discussed elsewhere A more significant omission—the renal and metabolic aspects of renal tubular acidosis (3)—is remedied by the report of Wrong and Davies (228) from this laboratory The interesting problem of renal diabetes insipidus (33 40 142a, 179) could not be discussed adequately without a detailed consideration of the recent physiologic observations of Wirz (223 224) but limitation of space forced the omission of this and certain other topics

RENAL AMINO ACIDURIA

Since the technic of paper chromatography was first applied to the analysis of urine (54) pathologic amino-acidura has been found in a host of conditions In some of these such as β aminoisobutyric aciduria (48) the finding is without clinical significance in others amino-aciduria has resulted from severe (73) but sometimes reversible (201) acute damage to the renal tubules Still others are of major clinical and metabolic interest, and it is with some of these that the following account is concerned Until recently distinction of a tubular origin for any particular amino-aciduria was uncertain. It depended on the finding of a normal plasma level of total α amino nitrogen together with a qualitatively normal amino acid chromatogram in the

normally leads to the virtually complete tubular reabsorption of this ion the continued excretion of sodium by a nephritic patient with *such a deficiency is generally taken to signify* failure of its tubular reabsorption These crude clinical approximations to the definition of a tubular defect are inadequate and often fallacious [4]

Wherever it has been possible to make quantitative studies of the active transport mechanisms of the renal tubule it has been found that the reabsorptive process has a limiting maximal rate (194 195) Although the demonstration of such reabsorptive maxima has been made by study of the whole intact kidneys it must be assumed that the reabsorptive capacity of each tubule is also individually limited In a population of nephrons known to be anatomically heterogeneous it is to be expected that tubular functions such as absorptive maxima *will vary from nephron to nephron* (29) *It becomes necessary there*fore to enquire whether certain renal phenomena commonly attributed to defective tubular function may not rather be phenomena of "glomerular tubular imbalance in which a functionally intact tubule is loaded beyond its absorptive capacity by virtue of its possessing an excessively filtering glomerulus

The concept of glomerular tubular imbalance has been applied with some success to the study of renal glycosuria (28 123 173) whereas some such cases may result from a metabolic fault in the tubular transport mechanisms there are others that can only be explained satisfactorily by heterogeneity of the nephron population (213) It has also been suggested that a similar mechanism may underlie the amino aciduria in some cases of the Fanconi syndrome without cystinosis (28)

The same problem arises in assessing functional disturbances in patients with organic renal disease leading to progressive destruction of the kidneys In chronic azotaemic renal failure there is some evidence that impaired osmolar concentrating capacity isosthenuria (167 168) and even the phenomenon of salt wasting (206) *may result from ex*cessive glomerular filtration into intact surviving tubules

With the recent emergence of precise chemical technics (55 77 150) it has become possible to define tubular disorders in which the reabsorption of either a single amino acid e g β aminoisobutyric aciduria (48 77) or group of amino acids e g cystinuria is defective These can be regarded as the pure tubular disorders which being genetically determined may reflect the absence of a single particular system of enzymes from the tubular cells they need not produce either structural or general functional disorganisation of the kidney

cystinuria in failure of the tubular reabsorption of cystine is thus established and it would appear from the observations of Dent and associates (63) that there is no disorder of the intermediary metabolism of the sulphur containing amino acids in this syndrome.

Evidence concerning the significance of the associated excretion of lysine arginine and ornithine has come from two quite different avenues of study. First Robson and Rose (178) have applied the concept of competitive inhibition in an experimental study of the renal excretion of these amino acids They elevated the plasma level of lysine by infusing L lysine (5 Gm /70 kg body weight) into normal subjects patients with cystinuria and "heterozygous carriers of recessive cystinuria" In the normal individuals and the heterozygous carriers the infusion increased the renal clearances of cystine arginine and ornithine as would be expected if the 4 amino acids share a common pathway of tubular absorption which was saturated by the increased filtered load of lysine In the cystinuric patients the infusion of lysine had no effect on cystine clearance as would be expected from its apparent prior identity with the rate of glomerular filtration Secondly Harris (96) has recently summarised in simplified form the many detailed observations that he and his collaborators have made on the urinary output of cystine lysine and arginine by the healthy relatives of cystinuric patients Among such relatives the output of cystine may vary from the normal to the frankly cystinuric level but throughout this range there is a close correlation between the output of cystine and that of lysine On the other hand abnormally increased excretion of arginine (and perhaps of ornithine) is found only when the output of cystine and lysine is high, an observation that Harris and co workers have taken to suggest that arginine and ornithine are absorbed preferentially to cystine and lysine The genetics of the disorder are complicated by the occurrence in different families of two patterns of recessive inheritance The original publications (97–99) must be consulted for the details but most instructive is the finding that homozygous individuals always show the complete syndrome by excreting all 4 amino acids whereas the incompletely recessive heterozygous (semicystinuric) subjects appear to have a less severe tubular lesion and excrete considerably less lysine and cystine together with normal quantities of arginine and ornithine This again supports the notion that the common absorptive pathway has a greater affinity for arginine and ornithine than for the other 2 amino acids

Cystinuria is unique in being the one renal amino aciduria in which

plasma ultrafiltrate This crude method sufficed to separate off the amino acidurias of overflow type such as phenylketonuria and that of severe liver disease in which the appearance of amino acids in the urine was due to increase in their levels in the plasma Evered (77) using ion exchange column chromatography has now measured the renal clearance of individual amino acids and he has also obtained amino acid clearances in patients with the adult Fanconi syndrome β aminoisobutyric aciduria and the Hartnup syndrome These new data in general confirm the views formed earlier on the basis of investigation with the qualitative and semiquantitative paper chromatographic methods

Two important topics—galactosaemia and the so-called Hartnup syndrome—have been deliberately omitted from consideration in the following account Any account of the first would merely echo the recent publications of the author s pediatric colleagues in Manchester (105–107 118 189) The full significance of the second also characterized as "hereditary pellagra like skin rash with temporary cerebellar ataxia constant amino aciduria and other bizarre biochemical features" is not yet apparent and it suffices to draw attention to the one excellent report so far written on the subject (12)

Cystinuria (Cystinelysinuria, Cystine Lithiasis)

In 1951 Dent and Rose (61) re examined the problem of cystinuria with renal calculus formation and for the first time drew a clear distinction between this genetically determined disorder and other conditions in which cystine is excreted in the urine They showed that, in addition to excreting quantities of cystine affected individuals excreted large amounts of lysine, arginine, and ornithine, and it was suggested that a single enzyme system which was deficient in cystinuric subjects was responsible for the renal tubular reabsorption of these 4 basic amino acids Stein (210) found that the average daily output in affected subjects was 0 73 Gm cystine 1 8 Gm lysine 0 83 Gm arginine and 0 37 Gm ornithine The subsequent and recent advances in the study of this syndrome have been both exciting and complex First, it was shown by polarographic measurement that the plasma level of cystine was normal or somewhat low (89) and later that the renal clearance of cystine in cystinuria was up to 30 times that of normal subjects the absolute values approximating closely to the measured (63) or assumed (178) glomerular filtration rate The origin of familial

cystinuria in failure of the tubular reabsorption of cystine is thus established and it would appear from the observations of Dent and associates (63) that there is no disorder of the intermediary metabolism of the sulphur containing amino acids in this syndrome.

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Cystinuria is unique in being the one renal amino aciduria in which

it is certain that the excretion of amino acids is directly harmful (There is no direct evidence to support the suggestion that chronic amino aciduria in other conditions may give rise to bone disease or to hepatic cirrhosis) Within the range of physiologic change in urinary pH, cystine has a solubility between 300 and 400 mg per litre (62), it tends to come out of solution when the rate of urinary flow is low and the urine acid These conditions obtain physiologically during the night (207) when the urine of cystinuric subjects is actually supersaturated with cystine (62) Since the urinary output of cystine *is determined solely by its plasma level and the rate of glomerular* filtration the only possible phophylactic therapy is to alkalinize the urine and maintain a high urinary flow by water drinking To be effective alkalinization must maintain the urinary pH as high as 7 6 (62) and this is therapeutically difficult Dent and Senior (62) recommend that patients should drink at least 3 litres of water daily taking 500 ml every 4 hours during the day and 750 ml in each 4 hours during the night In patients with calculi some dissolution may occur when the rate of urinary flow is high and the increased output of cystine will then produce a spuriously high cystine clearance It has also been suggested (62) that dissolution of cystine calculi may accompany the development of renal failure with its attendant fall in glomerular filtration and acquired polyuria

The patient with cystinuria is exposed to the same hazards as any *other calculous patient he is in danger of* pyelonephritis hydronephrosis and death as a result of progressive renal damage Knowledge of the genetics of the disease may allow earlier diagnosis and the earlier institution of prophylactic measures

Cystine Storage Disease with Amino Aciduria (Lignac Fanconi Syndrome)

Cystinosis cystine storage disease or the Lignac Fanconi syndrome with an incidence of approximately 1 case per 40 000 of the general population (18) has acquired a theoretic importance out of all proportion to its rarity It is a genetically determined disorder inherited as a simple mendelian recessive character (18) in which the primary defect is unknown but which may involve the general metabolism of proteins and amino acids (8 19) and which is characterised pathologically by the deposition of cystine in many tissues (22 92) Clinically

it has long been confused with cystine lithiasis and some still believe that the two conditions are related (92 165) However, most authorities now agree that cystinosis with amino aciduria and cystinelysinuria are separate entities (59) However the two conditions may rarely occur together by chance in the same family (22 59) and confusion is furthered by the occasional development of microliths of cystine in the kidneys of patients with cystinosis (8) Whereas cystine lithiasis is associated with the renal excretion of 4 basic amino acids the amino aciduria of the Lignac Fanconi syndrome is massive or generalised and is usually associated with renal glycosuria As many as 20 amino acids may be excreted in excess in the urine and characteristically among these are valine leucine phenylalanine tyrosine proline lysine serine cystine aspartic acid and threonine (19 22)

Fanconi (78) first proposed the concept of an innate defect of the renal tubules to account for his finding of renal glycosuria phosphaturia and the wasting of base in the urine The discovery of amino-aciduria by McCune and associates (134) extended the theory to include the defective reabsorption of amino acids (55 79 80 134) Dent (55) by using paper chromatography revealed the extent of the amino aciduria since the plasma level of total α amino nitrogen and the chromatogram of an ultrafiltrate of plasma were both normal, he concluded that the amino aciduria was of renal origin He has persisted in this belief and has shown that the renal clearances of "total α amino nitrogen" may approach the glomerular filtration rate in this syndrome (57 58) In contrast, others (19 225) have found plasma levels of α amino nitrogen increased by up to 100 per cent and they claim that the amino aciduria is of the "overflow" type. This dispute has not yet been resolved conclusively but in the genetically determined adult type of Fanconi syndrome it is now certain that the amino aciduria results from defective tubular reabsorption In such patients Evered (77) has measured quantitatively the plasma levels of some 21 individual amino acids by ion exchange chromatography and has found them within normal limits The total urinary output of amino acids by the same patients was 2 to 5 times the average normal, and the renal clearances of individual amino acids was greatly increased Robson and Rose (178) report a cystine clearance approximating the inulin clearance in another adult patient. The strongest support for the hypothesis of a renal origin of the amino-aciduria has thus come from study of adult patients and comparable

quantitative measurements of amino acid clearances in children with cystinosis are urgently needed In the meantime Bickel (17) who has been the principal protagonist of an extrarenal origin for the amino-aciduria now admits that a tubular defect must also be a factor

Although the genetic basis of the Lignac Fanconi syndrome is established beyond doubt, the evidence that the renal tubular disorder itself is innate remains conflicting The patient is usually quite well in the first 6 months of life and the characteristic disturbances of electrolyte and water metabolism may develop even later than this (22) *It has been shown recently that the amino aciduria is not present at* birth but develops somewhere between the fourth and sixth months (17) Since there is a physiologic amino aciduria in the newborn (20) *which is exaggerated in prematurity (68) Bickel s (17) recent observa*tion is of considerable importance It suggests that amino aciduria in the Lignac Fanconi syndrome may be acquired, in a fashion analogous to the acquisition of amino aciduria in galactosaemia

It is extremely difficult to rationalize these biochemical observations with the finding of apparently congenital anatomic abnormalities of the renal tubule By microdissection technic Clay and associates (39) discovered a short hypoplastic proximal tubule which in most cases examined (8 out of 11 up to January 1957 (52)) was converted in its proximal third into a thin and narrow "swan neck" Many observers have considered this anatomic abnormality to be the basis of the functional tubular defects but its significance becomes questionable with the recent finding of a similar swan neck in the proximal renal tubules of congenital familial nephrosis (52) a disorder in which generalised amino aciduria and glycosuria apparently do not occur Moreover in patients with the Lignac Fanconi syndrome dying early *in the course of the disease the renal tubules were histologically nor*mal (67 108)

Any satisfactory hypothesis for the renal manifestations of this syn*drome must explain the delayed development of amino aciduria* the fact that renal damage commonly progresses to a severe degree of renal failure and the claim *(22 182 191) that in the established case* prolonged treatment with alkalis and vitamin D may result in amelioration or disappearance of the glycosuria and amino aciduria As yet no single hypothesis can meet these requirements it would however seem most reasonable to ascribe the development of renal tubular disorder to a general metabolic disturbance of which cystinosis

storage in the reticuloendothelial system is another expression This would bring the Lignac Fanconi syndrome into the same group of secondary genetic disorders of the renal tubule as galactosaemia and Wilsons disease

It can be said with some confidence that no functional significance can safely be attached to the absence of phosphatase from the proximal renal tubules (44 212) This abnormality has certainly been seen in cases of classic cystinosis (8) but it is also a commonplace finding in many kinds of renal disease (8 136) The reduced phosphatase reaction in spleen liver and bone marrow (8) and the claim that the erythrocytes are abnormally permeable to phosphate (170) may be more significant If the claim is confirmed a clue may be provided to the fundamental nature of the primary metabolic defect

Much attention has been directed to the supposed occurrence of organic acidaemia in the Lignac Fanconi syndrome and Bickel and collaborators (8 19) have inferred that these organic acids may contribute to the production of renal damage Recognition of organic acidaemia depends on the indirect calculation of "undetermined anion" this is fraught with cumulative analytic error and doubtful physicochemical assumptions Moreover the development of an anion gap is met frequently in renal insufficiency of many different types and it is more likely that this finding in the Lignac Fanconi syndrome is a result rather than a cause of renal damage Until the matter has been adequately documented by identification of individual organic acids in the plasma this aspect of the syndrome will remain of doubtful significance

The amounts of glucose and amino acids excreted by patients with the Lignac Fanconi syndrome varies from case to case and is probably never sufficient of itself to promote an osmotic diuresis Yet all authorities include thirst and polyuria among the earliest and most prominent symptoms (22 92 134) and there is often impairment of osmolar concentrating capacity in the absence of conventional renal failure (Table 1) A metabolic acidosis is usually present, often of great severity and is commonly associated with a disproportionately high urinary pH continued excretion of bicarbonate and wasting of cation in the urine Each of these anomalies reflects additional tubular disorder in the one instance an impairment of the absorption of free water and in the other impairment of hydrogen ion secretion Detailed observations of renal function in these sick children have been too

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Any satisfactory hypothesis for the renal manifestations of this syndrome must explain the delayed development of amino-aciduria, the fact that renal damage commonly progresses to a severe degree of renal failure and the claim (22 182, 191) that in the established case prolonged treatment with alkalis and vitamin D may result in amelioration or disappearance of the glycosuria and amino aciduria As yet, no single hypothesis can meet these requirements it would, however seem most reasonable to ascribe the development of renal tubular disorder to a general metabolic disturbance of which cystine

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TABLE 1—POTASSIUM DEPLETION AND SECONDARY TUBULAR FUNCTIONAL DISTURBANCES IN FANCONI SYNDROME*

CASE No	AGE Mo.	POTASSIUM SERUM LEVEL	THIRST	POLYURIA[b]	URINARY OSMOLARITY[c] SP GR.	URINARY pH[d]	AMMONIA PRODUCTION
1	15	Low in 12-14 assays (as low as 2 1 mEq /L)	+++ 4 pt daily	Up to 3 2 L	Fixed 1 010-1 012	5 3 after $CaCl_2$	Increased with acidosis
2	18	< 3 8 mEq /L in 9-14 assays 2 9 mEq /L during glucose tolerance test EKG suggestive	+++	Up to 3 L	1 003-1 015	6 7	Normal or slightly raised
5	30	2 7 2 8 mEq /L in 2 assays	First word was "drink" 2 qt daily	Up to 2 L same after pitressin	1 001-1 004	6 2	Increased
6	15	3 3 mEq /L in 1 assay	+++	1-1 4 L same after pitressin	1 002-1 010	5 0	Increased
8	78	3 1 mEq /L or less in 7 assays EKG positive	3 pt daily at 3 mo drank enormously at 6 mo	Up to 2 2 L 1 3 L output at 0 39 L intake	1 005-1 010	6 1	Normal or increased

From clinical protocols of Bickel et al (22) and for case 6 from Bickel and Thursby Pelham (21)
a Figures refer to observed daily intake.
b Figures refer to measured daily urinary output
c Observed loss of osmolar concentrating capacity occurred in absence of continual renal ... tory failure although blood urea in each case was at times temporarily raised in patients with gross amino-aciduria normal blood urea is compatible with markedly reduced glomerular filtration rate (147 204)
d Data obtained at a time of severe spontaneous metabolic acidosis
e Normal or increased refers to ammonia coefficient it cannot be taken to mean an absolute increase in output as compared with normal.

few to permit any definite statement concerning the cause of these tubular defects In some patients the mechanisms of osmotic concentration appear to be congenitally defective (204) (*see* Fig 6)

It has become apparent recently that hypokalaemia, and presumably a potassium deficiency are frequent in this disease Pitressin-resistant hyposthenuria and defective hydrogen ion secretion are characteristic of the tubular disorder in potassium deficiency (*see* p 270) Data abstracted from the clinical protocols of Bickel *et al* (22) illustrate the association of potassium deficiency and these particular tubular lesions (Table 1) We believe that many of these defects could result directly from potassium deficiency the preservation of a relatively normal capacity for ammonium excretion in spite of submaximal urinary acidification would also be compatible with the effects of potassium deficiency However the general belief (134) that ammonium excretion is high in the Lignac Fanconi syndrome is unfounded When examined critically the output has sometimes been found to be subnormal (19 111 120) as would be expected in a disease associated with progressive renal damage Some of the described histologic changes in the renal tubules of patients with the Lignac Fanconi syndrome appear to be identical with the vacuolar nephropathy of potassium deficiency (*see* Plate 1) (8 94 134) It therefore seems certain that potassium deficiency contributes to the production of renal lesions in this syndrome and it may indeed be responsible for the progressive renal failure This begs the question of the mechanisms responsible for production of the potassium deficiency itself The author is not aware of any study which clearly documents this matter It is more than likely that anorexia coupled with renal loss is responsible and some data of Bickel and Hickmans (19) suggest that the renal clearance of potassium can be very high Whether a secondary increase of aldosterone production plays a part in facilitating the loss of potassium in these children is not known However in an adult patient with the genetically determined Fanconi syndrome and renal wasting of potassium Dr A H Gowenlock has found the urinary output of aldosterone to be 15 times the average normal value (204)

Childhood Disorders Simulating Lignac Fanconi Syndrome

There is a tendency at present to attach the label "Fanconi syndrome" or the more ponderous "nephrotic glycosuric aminoaciduric

dwarfism with hypophosphataemic rickets" to almost any condition in young children in which amino aciduria and glycosuria are associated with systemic upset and retardation of growth This is to be deprecated since the juvenile amino acidurias even in children with rickets, are by no means homogeneous Failure to demonstrate cystine in the cornea or in aspirated bone marrow does not exclude cystinosis which can only be eliminated by chemical analysis of the viscera and

TABLE 2.—POTASSIUM DEPLETION AND SECONDARY TUBULAR FUNCTIONAL DISTURBANCES IN NEPHROTIC SYNDROME*

	CASE 1	CASE [b]
Age	8	5
Potassium serum level	1 5–3 5 mEq /L before K therapy	Sustained hypokalaemia 2 3–2 4 mEq /L low exchangeable K paresis retention of administered K
Thirst	No comment	Constant
Polyuria	Very high diuresis often in presence of oedema	1 0–1 3 L even in presence of oedema
Urinary osmolarity sp gr	Not recorded	220–315 mOsm /L 440 mOsm /L after K therapy
Urinary pH	Alkaline in state of acidosis	6 8 when acidotic
Ammonia production	Low	5 mEq /day

Both patients were dwarfed osteoporotic and liable to attacks of tetany both had marked metabolic acidosis glycosuria and generalised amino-aciduria Clinical picture closely resembled Lignac Fanconi syndrome but neither patient had cystinosis Amino-aciduria in case 1 was reduced by alkali therapy Postmortem examination of case 2 revealed evidence of pyelonephritis in addition to type 2 nephritis (this secondary renal lesion may be a consequence of the potassium deficiency) bones showed evidence of osteomalacia.

[a] From Tegelaers and Tiddens (215)

[b] From Stanbury and Macaulay (205)

especially the spleen † (60) Nonetheless young patients have been described in whom characteristic renal functional disorders and various abnormalities of electrolyte metabolism were present but cystinosis absent (28 120 134 183 191) It seems likely that new and distinctive syndromes will be separated from among such patients and it is suggested that each case merits detailed study Already the so called Fanconi syndrome has been seen as a complication of glycogen storage disease (81) of vitamin D intoxication (219) of lead poisoning (36) and possibly of hepatolenticular degeneration (220) Recently 3 patients have been described in whom classic juvenile nephrosis

evolved into a clinical state closely resembling the Lignac Fanconi syndrome (205 215) as in that syndrome potassium deficiency has been implicated in the production of certain of the observed tubular defects (205) (Table 2) (A fourth very similar case has been described recently (107a)) The situation is further complicated by the finding in nutritional rickets of a distinctive pattern of renal amino aciduria which disappears after treatment with vitamin D (113) and by the occurrence of amino aciduria in coeliac disease which is often associated with rickets and dwarfism (22))

Finally metabolic acidosis and urinary abnormalities similar to those of the Lignac Fanconi syndrome have been described in association with a totally different clinical syndrome (130) The principal clinical features are mental retardation cataracts glaucoma and in some cases rickets Some or other of these clinical stigmata may be absent (21) and probably the condition is genetically determined (60) As with cystine storage disease this would seem to be a metabolic disorder in which the effects of an abnormal gene are clinically evident in the kidney and other tissues The occurrence of cataracts suggests an affinity with galactosaemia but nothing is yet known of the fundamental metabolic disturbance

The very multiplicity of primary disorders that can be associated with massive or generalised amino aciduria and glycosuria suggests that these may be relatively nonspecific indices of renal tubular damage Their occurrence adds indirect support to the hypothesis that amino-aciduria in the Lignac Fanconi syndrome may not be innate but an early acquired consequence of some innate generalised metabolic disorder

Fanconi Syndrome in Older Children and in Adults

Just as several distinct disorders in infancy have been called the Fanconi syndrome so in the adult and in older children the same term is applied to a heterogeneous mixture of clinical conditions Most of the patients to whom this diagnostic label has been attached have had late rickets or osteomalacia and hypophosphataemia together with renal glycosuria renal amino aciduria and probably renal phosphaturia A metabolic acidosis is usual but apparently not invariable (124 154) and when present it may be associated either with a somewhat reduced capacity for acid and ammonia excretion (5 111 182) or with gross impairment of this tubular function and resulting

dwarfism with hypophosphataemic rickets" to almost any condition in young children in which amino aciduria and glycosuria are associated with systemic upset and retardation of growth This is to be deprecated, since the juvenile amino acidurias even in children with rickets, are by no means homogeneous Failure to demonstrate cystine in the cornea or in aspirated bone marrow does not exclude cystinosis which can only be eliminated by chemical analysis of the viscera, and

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hyperchloraemia (59 147 192) It is often forgotten that glomerular filtration may be severely reduced as it may be in the infantile syndrome and insufficient attention has been given to progressive renal destruction as a factor contributing to the development of metabolic acidosis (120) Less common accompanying disorders include hypokalaemia with renal wasting of potassium (74 147 183 192) low serum levels of urate (147, 154) with renal uricuria (192) and impaired osmolar concentrating capacity or renal diabetes insipidus (204)

Among these adult patients are some in which evidence of renal involvement can be traced back to early life (204) and in which a genetic origin is likely (60 204) but they differ from the Lignac Fanconi syndrome in having no deposits of cystine in the viscera These patients may have suffered from thirst and polyuria in early childhood (183 204) and although there may be osseous involvement at that time it may be minimal and easily overlooked or manifest only in some degree of growth retardation Osteomalacia (60 204) evidence of hepatic cirrhosis (212) or renal failure develop in early adult life

Another group of patients come to attention for the first time in middle age with symptoms of osteomalacia and varying combinations of tubular defects (60 102 124 147) In many of these cases no causative factor has been identified but in others a genetic origin is established and like the Lignac Fanconi syndrome the disease is inherited as a simple mendelian recessive (59 60) In addition to their sharing this common pattern of inheritance it has been claimed that both the infantile and adult cases exhibit the swan neck deformity of the proximal tubule (39) but no adult has been found with cystine storage disease (60) The adult and infantile i e Lignac Fanconi syndrome types appear to be clearly distinct each runs true to type within a given family and cystinosis has not been seen in the infantile relatives of the genetically determined adult Fanconi syndrome (60)

It has always been thought that among the sporadic adult cases of this syndrome there may be some in which the multifactorial tubular defects develop as a result of some form of primary renal disease Actual proof of this happening has been lacking until recently when characteristic features of the Fanconi syndrome have been described in adults with the nephrotic syndrome (26 27) and with myelomatosis (74 192) The author is aware of another myelomatous patient with renal glycosuria and amino aciduria (190) and the cases in children with nephrosis to which reference has already been made may come into the same general category (205 215) Any attempt to interpret

these complications of renal disease with gross proteinuria is necessarily speculative On the one hand all these patients had gross disorders of protein metabolism and possibly severe protein deficiency, and the effects of such on the renal tubule are unknown On the other hand it might be argued that the renal tubules were reabsorbing excessive amounts of filtered protein or even of abnormal myeloma protein and that this may initiate the sequence of cytoarchitectural events described by Oliver and co workers (159 161) as occurring in the proximal tubules of rats with proteinuria following the injection of protein Since the principal changes noted by them involved the apparent disorganisation of the mitochondria—the cellular locus of energy production—some disturbance of reabsorptive function might reasonably be expected this has been discussed briefly elsewhere (171) The finding of crystal like inclusions in the renal tubular epithelium of the myelomatous patient of Engle and Wallis (74) is of the greatest interest in this respect the author is aware of another case of myeloma with similar findings (27) The development of amino aciduria in children with nephrosis appears to be not uncommon (110 226) a deliberate study of its evolution should throw further light on these problems (205)

Osseous Disorders Complicating Multifactorial Tubular Defects

Frequently it is the development of rickets or osteomalacia that brings the patient to clinical attention and leads to the diagnosis of a multifactorial tubular defect or of the Fanconi syndrome The osseous lesions are usually ascribed to the low serum level of inorganic phosphorus which is considered to result from loss of phosphorus in the urine This is an undue simplification Among different patients of this heterogeneous group at least three distinct mechanisms may contribute either alone or in combination to the production of negative external balances and low serum levels of calcium and phosphorus These are defective absorption of calcium and phosphorus from the intestine (19 204), excessive excretion of phosphorus in the urine (124) and an abnormally high renal output of calcium (60 102 183)

It has yet to be proved that renal phosphaturia is innate in any of the conditions designated as Fanconi syndrome In the specific instance of the Lignac Fanconi syndrome in which many workers consid

hyperchloraemia (59 147 192) It is often forgotten that glomerular filtration may be severely reduced, as it may be in the infantile syndrome and insufficient attention has been given to progressive renal destruction as a factor contributing to the development of metabolic acidosis (120) Less common accompanying disorders include hypokalaemia with renal wasting of potassium (74 147 183 192) low serum levels of urate (147 154) with renal uricuria (192) and impaired osmolar concentrating capacity or renal diabetes insipidus (204)

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clearances to normal after correction of acidosis (192) or following the administration of alkalis and vitamin D (147) would favour this explanation

In a small group of patients with cystinosis Bickel and Hickmans (19) found no evidence of excessive phosphate excretion in the urine they attributed the development of rickets to impaired absorption of calcium and phosphorus from the intestine The author has obtained results agreeing with their hypothesis in the genetically determined Fanconi syndrome of early adult life (Fig 1) in this patient, the lesions of osteomalacia healed during therapy with dihydrotachysterol without appreciable elevation of the low serum phosphate level (204) This particular metabolic lesion which leads to inadequate utilisation of dietary calcium and phosphorus appears to be essentially the same as that which occurs in azotaemic renal osteodystrophy (128 202 203) The mechanisms responsible for defective alimentary absorption have not yet been defined but in azotaemic renal osteodystrophy it has been suggested renal failure leads to an acquired vitamin D resistance which can be overcome by the use of sufficiently high doses of calciferol (202) or dihydrotachysterol (128 203) The apparent occurrence of a similar metabolic lesion in nonspecific azotaemic renal failure (128 203) cystinosis (19) nephrosis (72 205) and the adult Fanconi syndrome (204) suggests that a single responsible factor may be common to all The only common feature that is immediately obvious is the presence of renal disease with destruction of renal tissue whatever its nature this factor may be as important for the production of osseous changes as are phosphaturia and calciuria

In most children with cystinosis even when there is a severe metabolic acidosis and an impaired capacity for urinary acidification the renal output of calcium is relatively low (19 111) and it cannot be considered to contribute to the development of rickets In other patients with multifactorial tubular defects either genetically determined (60) or of uncertain origin (102 183) the renal output of calcium has been high as in the syndrome of renal tubular acidosis (3) it is possible that calciuria plays some part in the production of defective skeletal mineralisation Some of these patients do in fact, have "renal tubular acidosis" as one of their many tubular disorders (59 147 192) But, although it has been demonstrated that correction of acidosis in the adult Fanconi syndrome results in a reduced urinary output of calcium (183) it has not been proved that the administration of alkalis alone would correct the osteomalacia Indeed in the litera

er phosphaturia to be invariably present Bickel (17) has found both the plasma level and the urinary output of phosphorus to be normal during the early months of the disease) Gross renal phosphaturia has however, been demonstrated in some older children (120 case 2) and adults (44 147) with multifactorial tubular defects, in 2 patients with

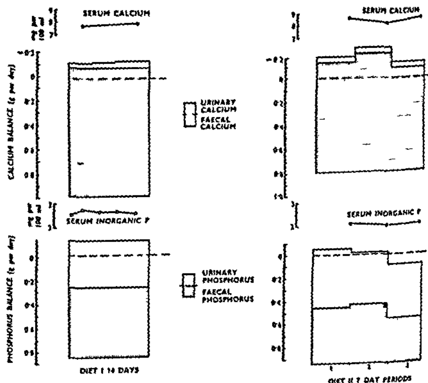


Fig 1 —Calcium and phosphorus balance data in patient with adult Fanconi syndrome With two entirely different diets calcium and phosphorus absorption from bowel was defective during treatment with dihydrotachysterol calcium and phosphorus were retained in ratio of 2 1 and the osteomalacia healed without a return of the serum phosphate level to normal (From Stanbury [204])

normal glomerular filtration rates the phosphate T_m was significantly reduced (44 124) In other, essentially similar patients both older children (111 120 case 1) and adults (5 212) there appeared to be no demonstrable phosphaturia This suggests that impairment of phosphate reabsorption is present irregularly and perhaps acquired as a secondary consequence of metabolic acidosis hyperparathyroidism or even of potassium depletion The reversion of high phosphate

to be a hyperchloraemic acidosis associated with the excretion of neutral or slightly alkaline urine It thus seems possible that a specific intoxication can give rise to all the clinical features of the Fanconi syndrome

Future investigation into the effects of heavy metals may prove critical to the understanding of the many clinical and theoretical problems posed by the multifactorial tubular disorders At present, it is possible only to indicate a few tenuous or potential links between totally different investigations At the one extreme we have the clinical experience of Chisolm *et al* and the distinct probability that osteomalacia has developed in a few patients (45 220) with the chronic copper intoxication of Wilson s disease On the other hand the observations of Clarkson and Kench (38) suggest that it may be possible to distinguish specific pathways of amino acid absorption by their differential sensitivity to heavy metal ions It is tempting if speculative to seek a connexion between the observation that the renal tubule is particularly sensitive to cadmium ion (38) and the clinical fact that an apparent osteomalacia may develop in men with chronic cadmium intoxication (122 156) The recent claim (34) that the liver protein of patients with Wilson s disease differs from the normal suggests another possible and distant link between cadmium intoxication and hepatolenticular degeneration The proteinuria of cadmium intoxication (197) may not be primarily of renal origin, but a result of the circulation and glomerular filtration of proteins of low molecular weight (196) Smith and Kench (196) take this to suggest an interference by cadmium with the hepatic synthesis of proteins This provokes the speculation that the oligopeptides excreted in the urine in Wilson s disease (216 218) may originate in a comparable way

Wilson s disease although it is a genetically determined disorder is included among the effects of heavy metals since there is little doubt that its various clinical features result from intracellular deposition of copper It is inherited in an autosomal recessive fashion (13 142) and the primary metabolic defect is not precisely known The plasma concentration of the specific copper binding protein, caeruloplasmin is greatly reduced (185) and it has been suggested that there is a primary failure of the synthesis of this protein Why such a deficiency should lead to an apparent increase in the absorption of dietary copper (141 230) is by no means clear but retention of copper and its deposition in tissues produces hepatic cerebral, and renal abnormalities

Amino aciduria in patients with hepatolenticular degeneration was

ture on pure renal tubular acidosis the author has been unable to find a single documented instance of cure of osteomalacia by means of alkali therapy alone All cured cases appear to have received large doses of vitamin D This suggests the possibility that the homeostatic defect—the failure of base conservation—could be less important in the production of osteomalacia than the unknown factor to which reference has been made previously

The author has permitted himself this digression to suggest that insufficient attention has been given to the causation of the rickets and osteomalacia associated with renal tubular disorders and to plead for a more critical attitude to this problem To demonstrate the presence of hypophosphataemia or of renal phosphaturia is not to establish the cause of defective mineral deposition in bone

Functional Renal Tubular Disorders Apparently Caused by Heavy Metals Poisoning

Experimental intoxication of animals with the salts of uranium and mercury has long been used for the production of an acute renal necrosis in which damage is largely restricted to the proximal tubules (133 71) In human poisoning with corrosive sublimate epithelial necrosis occurs predominantly in the proximal tubule (15 160) In children dying of lead poisoning nuclear inclusions giant nuclei, and fatty vacuolation have been described in the cells of the proximal renal tubules and of the looped tubules (25 140) the collecting tubules remaining apparently healthy Recent observations (38) indicate that less severe intoxication with mercury and lead and more especially with uranium and cadmium may induce functional disorders of the renal tubules Arguing by analogy with the histologic findings in more severe intoxications it may be inferred cautiously that these functional defects involve the proximal tubule Glycosuria, apparently of renal origin was recorded in chronic lead poisoning by several earlier workers (*see* references in 38 95) and more recently the occurrence of amino aciduria has been recognised (140 221) Cystine taurine and especially β aminoisobutyric acid were found in the urine in excess while the plasma level of total α amino nitrogen was normal (221) More extensive tubular disorders were present in a single case of lead poisoning described by Chisolm *et al* (36) This child in addition to having glycosuria and amino aciduria was also rachitic and had a low serum level of phosphate and there appeared

renal tubular cell The latter hypothesis remains unproved on the other hand it is sometimes possible to demonstrate a reduced tubular reabsorption of phosphate in patients who are excreting normal amounts of phosphorus in the urine (180) The tubular reabsorption of phosphate appears to be influenced by so many extrarenal factors that it may at times be impossibly difficult to establish the cause for a given instance of renal phosphaturia

It has been assumed apparently on the basis of observations made originally on a single subject (186) that phosphate reabsorption at high plasma levels of phosphate is limited by a stable maximum rate (T_m) as it is in the dog (7) Smith (194 195) suggests that when the dietary intake is normal the plasma level of phosphate is such that the load of phosphate filtered is near to the maximal rate of reabsorption Recent observations on young adults (6) in which meticulous care was taken to maintain basal and constant conditions of study support the concept of a stable T_m Similar and equally careful experiments (129) in which conditions were *not* basal, revealed wide variations of T_m in the same subject phosphate reabsorption appeared to vary with the rate of glomerular filtration and the authors explain their results in terms of the perfusion of unpredictably varying numbers of nephrons These two groups of observations are of the utmost importance for they underline the fundamental difficulties involved in assessing the renal handling of phosphate in disease In placid patients it may be possible to make observations under reproducible and basal conditions by the use of these technics it has been claimed that the phosphate T_m of postmenopausal women is regularly reduced following the administration of oestrogens (155) But in most patients and especially in children the emotional effects of the investigative procedure and the infusion of phosphate salts itself are likely to influence renal haemodynamics in varying degree and the results obtained are correspondingly difficult of interpretation Effects on renal haemodynamics and accompanying changes in phosphate T_m are clearly evident in the experiments of Rupp and Swoboda (180 181) which were designed to explore the action of vitamin D in children with refractory rickets Similar haemodynamic effects produced when crude extracts of parathyroid gland (parathormone) are administered intravenously may account for the continued dispute as to whether or not the parathyroid glands influence phosphate excretion by varying the reabsorption of phosphate by the tubules This question is discussed by Nordin in this

first recorded by Uzman and Denny Brown (217) and its probable origin in defective tubular reabsorption was suggested by the later observations of Cooper *et al* (45) and Matthews *et al* (142) A renal tubular origin has now been proved by the demonstration of massive amino aciduria in patients with a plasma pattern of amino acids that is both qualitatively and quantitatively normal (211) This renal defect is presumably nonspecific as with poisoning by other metals or with the intracellular accumulation of galactose I phosphate or glycogen Some genetically or clinically involved individuals may not exhibit amino aciduria and one may perhaps infer that the tubular disorder will progress as accumulation of copper increases A few patients have been found to have renal glycosuria (45) others may have impairment of urate reabsorption and attention has already been directed to the occasional occurrence of apparent osteomalacia A systematic survey of discrete renal functions in a group of patients with Wilson s disease has been reported recently (14a)

Many aspects of this disease remain obscure The paradoxic occurrence of copper retention relatively low plasma levels of copper and an increased urinary output of copper has not yet been fully explained Matthews *et al* (142) found a positive correlation between the urinary output of copper and the excretion of amino acids They inferred that there may be an impairment of the renal tubular reabsorption of filtered copper a hypothetic moiety of plasma copper that was not bound to protein and consequently filterable at the glomerulus This factual observation has been confirmed (14) and increasing knowledge of the state of plasma copper in this disease is providing some direct support for the hypothetical formulation Uzman (216) and Uzman and Hood (218) believe that copper is filtered in a chelation complex with oligopeptides which remain unabsorbed in the tubule and carry copper to the urine in this form

RENAL PHOSPHATURIA

The term "renal phosphaturia" is widely and indiscriminately used to imply a state of affairs in which excessive amounts of phosphorus are excreted in the urine through impairment of the reabsorption of phosphate by the renal tubules In certain genetically determined disorders it has been further inferred that the impaired phosphate reabsorption is based on a deficiency of some unspecified system of enzymes in the

renal tubular cell The latter hypothesis remains unproved on the other hand it is sometimes possible to demonstrate a reduced tubular reabsorption of phosphate in patients who are excreting normal amounts of phosphorus in the urine (180) The tubular reabsorption of phosphate appears to be influenced by so many extrarenal factors that it may at times be impossibly difficult to establish the cause for a given instance of renal phosphaturia

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volume on the basis of observations made on hypoparathyroid patients (101 144) the author will assume that a tubular effect of parathyroid hormone is established

In view of the difficulties experienced in investigating the renal excretion of phosphate by normal subjects it is not surprising that clinical appraisal of the same problem has given rise to considerable confusion The following indices of the renal handling of phosphate have all been used by different workers in the clinical field

(1) The daily output of phosphorus in the urine (82 180 181)

(2) The renal clearance of phosphate as determined in short urinary collection periods (53 174)

(3) A 24 hour clearance calculated from the measured daily output of phosphorus in the urine and a single plasma level of phosphate obtained under varying and arbitrary conditions (65 139 180 181)

(4) A phosphate excretion index in which the reabsorption of phosphate is related to the simultaneous filtration rate of phosphate (35 47 125 147 157) The ratio of clearance of phosphate to glomerular filtration rate provides an index of the fraction of filtered phosphate which remains unabsorbed by the tubules As originally used by Lambert (124 125) and by ourselves (147) the normal values were determined over a wide range of plasma phosphate levels and abnormal data were compared with this established normal Others (158) by applying an incompletely valid mathematical treatment to the same data, have derived a convenient index which measures the deviation of an observed clearance ratio from the normal value expected with any particular level of plasma phosphate

(5) A somewhat similar index of tubular activity is provided by measurement of the absolute amount of phosphate reabsorbed per minute per 100 ml of glomerular filtrate formed (11 66) If this were measured at plasma levels that saturate the absorptive capacity of the tubules (181) it should minimize the effects of changing filtration rate on phosphate T_m

(6) Measurement of the classic phosphate T_m that is the absolute minute rate of phosphate reabsorption when the reabsorptive capacity has been saturated by artificial elevation of the plasma phosphate concentration

It may seem inappropriate to discuss methods of measurement in a review of tubular disorders but supposed renal phosphaturia frequently features in the description of these conditions and it is so

often diagnosed on inadequate grounds that some appraisal must be attempted

The first of the above indices is clearly of little value unless the dietary intake of phosphorus is stabilised and appropriate correction is applied for the varying weight or surface area of the subjects compared even constancy of intake does not ensure constant absorption of dietary phosphorus Nonetheless the urine of normal individuals becomes virtually free of phosphate when the serum phosphate falls below about 2 2 mg per 100 ml (147) continued output of significant amounts of phosphorus then constitutes presumptive evidence of impaired renal conservation

The second index, although it can be measured easily and accurately may provide the most misleading information It is remarkable that a value of 4 to 6 ml per minute for the clearance of phosphate at normal plasma levels which was presented originally without supporting data (44) should be quoted uncritically in the clinical literature dealing with renal tubular disorders In infants children and adults the phosphate clearance varies extremely (53 174) and in the same individual the clearance may vary through the day and from day to day (53) Even within the normal range of plasma phosphate levels the clearance varies with the plasma level (125) (Fig 2) The difficulties involved in obtaining a representative phosphate clearance can be illustrated by reference to Figure 2 which shows the regular but relatively enormous diurnal changes in the output and plasma level of phosphate These data confirmatory of others published earlier (207) refute the opinion of Ollayos and Winkler (162) that the diurnal changes in phosphate excretion are unrelated to changes in the plasma phosphate level Parenthetically some investigations of the phosphaturic action of parathormone have been made in the early morning at this time a spontaneous increase in the phosphate clearance is to be expected (Fig 2) and its occurrence can be detected in some of the published data (e g 117) From Figure 2 it is evident that there may be a threefold change in the clearance of phosphate within a single day and the average value is much higher than the supposedly normal figure quoted above The glomerular filtration rate was not measured during the experiments recorded in Figure 2 but the output of creatinine fluctuated only slightly and previous experience (193 207) indicates that the glomerular filtration rate of resting subjects varies little during the day It follows that the fourth index the clearance ratio

(phosphate clearance/glomerular filtration rate) will also change greatly in the course of the day and this figure is equally valueless unless related to the plasma level at which it was obtained (125 147 158) It may also be inferred from Figure 2 that the use of short urinary collection periods for calculating the clearance ratio has little advantage over the use of an "integrated" or "24 hour" clearance The

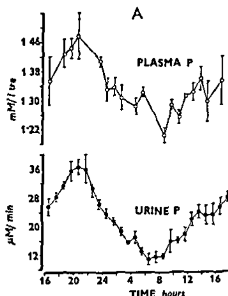


FIG 2—Spontaneous changes of plasma phosphate level and of urinary output and renal clearance of phosphate during 24 hour period in 3 normal young adults resting and taking a small constant amount of fluid each hour Urine was collected at hourly intervals and blood taken at midpoint of each clearance period Subject 1 • subject 2 ○ subject 3 ○ (From unpublished data of Mills and Stanbury [148 149]) A Mean and S E of mean of the 3 subjects hour by hour *(continued)*

author calculates a 24 hour clearance of phosphate and creatinine from their respective daily outputs in the urine and plasma levels determined on a single blood specimen which is taken at midday 4 to 5 hours after the last meal or medicament When it is used in the course of metabolic studies in which the patient is receiving a fixed dietary intake the 24 hour phosphate clearance/glomerular filtration rate ratio can provide a valuable index of changes in tubular activity (139) When used for comparison in this fashion the error that may occur from using the clearance of creatinine as a measure of glomerular filtration is minimized

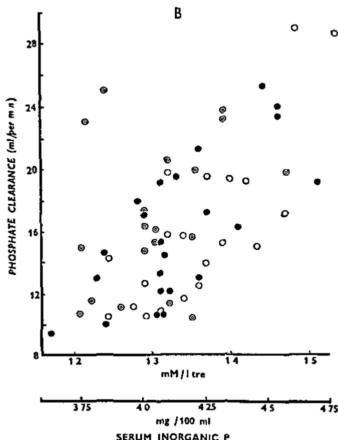


FIG 2 (*cont*)—*B* individual hourly clearance data related to corresponding plasma levels note increase of renal clearance with increase of plasma level Mean of hourly clearances was for subject 1 16 0 ml/min for subject 2 16 5 ml/min for subject 3 17 0 ml/min. Corresponding 24 hour clearances calculated from 24 hour urinary output of phosphorus and serum phosphate value at midday were for subject 1 15 2 ml/min for subject 2 17 0 ml/min for subject 3 16 9 ml/min The two grossly aberrant values of subject 2 the first of the series in this subject emphasize further the fallacies involved in using short collection periods

There is little room for dispute that measurement of phosphate T_m and calculation of the saturation thresholds as suggested by Anderson (6) provides the theoretic ideal in investigating disorders of the tubular handling of phosphate The discomfort of the procedure and the experiences of Longson *et al* (129) militate against its general clinical use furthermore the infusion of large amounts of phosphate into pa

tients with osteomalacia may give rise to severe tetany There are two other theoretic disadvantages to its use (1) The prolonged infusion of phosphate may itself appear to depress the measured phosphate T_m (103 143 160) an effect that may possibly be related to change in either the plasma level or body content of potassium (103) or to an induced change of parathyroid activity (2) Certain factors may depress phosphate reabsorption at normal or slightly elevated plasma phosphate levels without having a demonstrable effect on phosphate T_m this appears to be the case with the phosphaturic effect of metabolic acidosis (186) Until these various difficulties have been circumvented there is much in favour of the continued clinical use of the third and fourth indices listed above

The above considerations apply to the exploration of possible renal phosphaturia in patients without organic renal disease as for example in the so called phosphate diabetes of refractory rickets Further difficulties arise in the presence of destructive renal disease It is not known whether reduction of glomerular filtration and progressive loss of nephrons affects the handling of phosphate by the surviving renal tubules (Table 3) Conceivably measurement of the ratio of phosphate T_m/glomerular filtration rate would provide a suitable basis for assessing phosphate reabsorption in this circumstance but no data are available either for renal disease in general or for those apparently phosphaturic disorders in which progressive renal damage is the rule (e g the Lignac Fanconi syndrome) It is also probable that the range of the clearance ratio established for normal individuals (125 147 157 158) is not strictly transferable to patients with renal disease and significantly reduced glomerular filtration This may account for the difficulties experienced when the clearance ratio is used in an attempt to detect primary hyperparathyroidism in patients with renal calculi (135) In the face of these uncertainties it may be advisable to resort to the indirect method of observing the effects on the phosphate clearance of eliminating influences which could be responsible for phosphaturia By this means it has been possible to demonstrate that a severe deficiency of potassium was responsible for phosphaturia in a patient with chronic pyelonephritis (139) (*see* Fig 5) and that the accompanying metabolic acidosis accounted for the increased phosphate clearance in the syndrome of renal tubular acidosis (9) and in a case of the adult Fanconi syndrome associated with myelomatosis (192) Assessment of the part played by *secondary hyperparathyroidism* in producing apparently high phosphate clearances in renal disease is

much more difficult and uncertain Methods used in testing parathyroid activity have recently been reviewed (35)

It is probably only in the condition of refractory or vitamin D resistant rickets and osteomalacia that the presence of renal phosphaturia

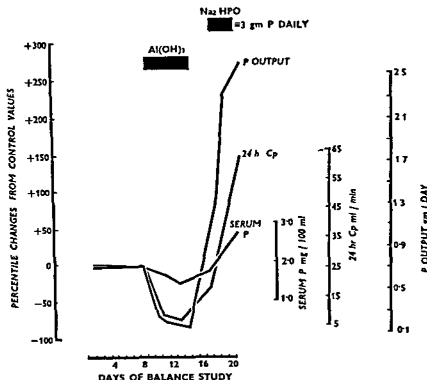


FIG 3—Effects of phosphate deprivation (aluminium hydroxide gel, 120 ml. daily) and of administered phosphate in 56 year-old male patient with vitamin D resistant osteomalacia proved by bone biopsy 11 of patient s relatives known to have same genetically determined disorder Full metabolic data in Stanbury and Lumb (208)

has been established by all the criteria discussed above Even in this genetically determined syndrome (60 215a) in which the serum phosphate level is usually very low and the urinary output of phosphorus relatively high it has not been proved that the renal tubules are primarily at fault, and the fundamental nature of the disorder remains undecided Three hypotheses have been proposed to account for the diminished tubular reabsorption of phosphate in refractory rickets

(1) there is an innate defect of the mechanism responsible for phosphate transport in the tubules (56 82 91 176) (2) it results from some innate disturbance of vitamin D metabolism (10 60) (3) it is an effect of secondary hyperparathyroidism (1 2)

When it has been measured the glomerular filtration rate in refractory rickets has been found normal (82 114 126 180 181 208) and the patients exhibit no evidence of primary renal disease Fanconi and Girardet (82) used the term "phosphate diabetes" for the syndrome and with others (56 102 111) they believe that renal glycosuria, refractory rickets and the Fanconi syndrome constitute a group of related and innate disorders of the proximal renal tubule There is much to commend this hypothesis for some patients with refractory rickets or osteomalacia have renal glycosuria (126) and others have amino-aciduria with or without glycosuria (60 77 121) However there are certain objections to this concept If the tubular transport mechanism for phosphate is congenitally defective it remains nonetheless responsive to physiologic stimulation and on deprivation of phosphorus or following its administration the pattern of tubular response is qualitatively the same as normal (Fig 3) When aluminum hydroxide gel was given all the dietary phosphorus remained in the faeces renal conservation was effected by a fall in phosphate clearance and the decrease in serum phosphate was only minor When additional phosphorus was given most was absorbed from the bowel and the increase in its urinary excretion was through an increase in renal clearance Creatinine output remained virtually unchanged Moreover the presence in the tubules of the cellular components necessary for phosphate transfer is shown by the frequent fall of the phosphate clearance to normal after massive vitamin D therapy (Table 3) (132 176 180 181) and by the fact that normal values of phosphate T_m may be achieved after such treatment (181) Robertson *et al* (176) have tried to explain this therapeutic response by postulating "a functional defect of an enzyme system of which Vitamin D forms the prosthetic group and which is responsible for the absorption of phosphate from the intestine and its reabsorption from the glomerular filtrate It seems possible to produce an adequate amount of the enzyme through the mass effect of large amounts of the vitamin" In criticism of this hypothesis it must be insisted that there is no demonstrable defect of phosphate absorption from the bowel (Fig 3) (208 222) although the absorption of dietary calcium is impaired (60 132 208 229) This failure of calcium utilisation, and the extremely low urinary output of calcium with

which it is usually associated, mimics precisely the findings in nutritional rickets when massive doses of vitamin D produce a satisfactory therapeutic effect, the metabolic response is the same as that which accompanies the healing of nutritional rickets (60 184 208) There is little doubt, therefore that interference with the action of vitamin D is responsible for certain facets of this syndrome the intimate nature of the interference however remains unknown As we have previously

TABLE 3—*Effects of Vitamin D in Syndrome of Vitamin D Resistant Osteomalacia* (208)

Date	Serum Levels of			Clearances of		Clearance Ratio P Cr
	Calcium, mg./100 ml.	Phosphate mg./100 ml.	Creatinine mg./100 ml	Phosphate ml./min	Creatinine ml./min	
2/27/57	8 9	3 7	0 79	16 3	73	22 3
3/13/57	8 8	3 3	0 74	17 8	72	24.2
100 000 units calciferol daily started 3/13/57 increased to 300 000 units daily 3/23/57						
3/26/57	9 6	4 3	0 85	11 4	65	17 4
4/ 2/57	10 3	5 2	0 90	8 9	50	18 0
4/ 9/57	12 2	5 3	1 26	10 8	39	28 9

P tient was 56 y ld wom n h ght 51 7 n. p n 53 7 weight 124 lb B n b opsy rev aled oste p osis with m n d grees of osteomala ia 4 f p t nt bl ngs had t bial f m ral osteotom es in hildhood youth. During observat n period p ti nt was rec ving a nsta t d et onta n ng 0 94 Gm. C 1 Gm. P Th 24 h ur ren l clearan es w re calculated as des ribed n text

N te th t *(1)* rum P was h gh th n is usu l n this syndrom poss bly rel t d to postmen p us l osteop rosis *(2)* first v lues for cl rance r t w t highest lim t of n rmal *(3)* ti l fall in P cl r n was pp ntly du to n reased tubula reabsorption nd the th rd nd f u th cl t os e normal *(4)* vit m n D nt xi t n led to hyp cal m a and gn fi ant renal d m g s th t n p ecise sign fican e can be attached to fifth l n t

pointed out (147) the phosphate clearance may be greatly increased in nutritional osteomalacia and as in rachitic dogs (100) vitamin D administration produces a fall toward normal values The analogous effects of high doses of vitamin D on the phosphate clearance in refractory rickets provide further evidence suggesting a probable extrarenal origin of the renal phosphaturia In this context, it must be remembered that large doses of vitamin D may reduce the glomerular filtration rate in refractory rickets (177) and that a fall in phosphate clearance due to increased tubular reabsorption of phosphate must be distinguished from that produced by renal damage resulting from vitamin D intoxication (Table 3)

The occasional occurrence of glycosuria and amino-aciduria in pa

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TABLE 3 —EFFECTS OF VITAMIN D IN SYNDROME OF VITAMIN D RESISTANT OSTEOMALACIA (208)

DATE	SERUM LEVELS OF			CLEARANCES OF		CLEARANCE RATIO P Ca %
	C l um mg /100 ml	Phosph te mg /100 ml	C tinine mg /100 ml	Phosphate ml /m n	C t n ml /m n	
2/27/57	8 9	3 7	0 79	16 3	73	22 3
3/13/57	8 8	3 3	0 74	17 8	72	24 2
100 000 units calciferol daily started 3/13/57 increased to 300 000 units daily 3/23/57						
3/26/57	9 6	4 3	0 85	11 4	65	17 4
4/ 2/57	10 3	5 2	0 90	8 9	50	18 0
4/ 9/57	12 2	5 3	1 26	10 8	39	28 9

Pat nt was 56 y r ld w m n h ght 51 7 n pan 53 7 n we ght 124 lb Bone b p y evealed osteop osis w th m n r degrees of osteom lac 4 of p t nt s siblings had t h al o f m l oste t m es n ch ldhood youth During observat n p od patient was re e ving a nsta t d t c nta ning 0 94 Gm. Ca 1 Gm. P The 4 hou renal cl n es w re cal ulated as des ribed n t xt

N te th t (1) um P was h gh r th n is usual in this syndrom pos bly r l ted t postm n p usal oste p osis *(2)* first v l es f l arance rati w r at highest l m t f n rm l *(3)* in ti l f ll in P l ran e was pp rently d e to reased t bular r be p tion and th th d nd fou th cle n t os a normal *(4)* v tam n D int x c t on led t hyper al em a d sign fi nt renal dam g so that n p cise ignificance can be atta h d to fifth l a n e rat

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The occasional occurrence of glycosuria and amino aciduria in pa

tients with refractory rickets provides at first sight, the strongest support for a primary renal defect in this disease? In 1 case however, glycosuria developed late in the course of the illness (10) an occurrence not readily explained in terms of an innate renal glycosuria Moreover Dent and Harris (60) mention the disappearance of amino-aciduria in 1 patient after vitamin D treatment Attention has already been directed to the disappearance of glycosuria and amino aciduria after vitamin D therapy in patients with the Lignac Fanconi syndrome and to the reversible amino aciduria of nutritional rickets Although the pattern of amino aciduria is different in these 3 distinct syndromes the observed response to vitamin D suggests that it may play some role in the tubular reabsorption of substances other than phosphate A critical step in the further elucidation of the nature of the renal defect in refractory rickets would be the quantitative study of amino aciduria and glycosuria preferably by saturation technics before and after treatment with vitamin D There is also scope for observation of the evolution of the renal disorders in a manner similar to that used by Bickel (17) *in his neonatal observations on patients with the* Lignac Fanconi syndrome

The possibility remains that in refractory rickets the renal phosphaturia reflects a state of secondary hyperparathyroidism This view originally proposed by Albright and associates (2) and subsequently championed by Albright and Reifenstein (1) is extremely difficult to confirm or refute Since glycosuria and amino aciduria do not appear to develop in primary hyperparathyroidism functional hyperparathyroidism would not readily account for the occasional presence of these abnormalities in refractory rickets Surgical exploration has demonstrated that there is both parathyroid hyperplasia and parathyroid hyperfunction in this syndrome as in the renal osteodystrophy syndrome (202) Linder and Vadas (127) removed 1 hyperplastic gland weighing 850 mg Removal of a single hyperplastic gland from the patient observed by Albright *et al* (2) was followed by a rise in serum phosphate a fall in serum calcium and the clinical development of tetany Moreover in the case reported by Lievre *et al* (126) the description of the roentgenographic osseous changes and the appearance of the single roentgenogram suggest that osteitis fibrosa was present, as well as osteomalacia Here again the analogy with certain kinds of vitamin D deficiency is close the changes of osteitis fibrosa may occasionally be grafted on to the osteomalacia which complicates the mal

absorption syndrome (50 204a) Whether a deficiency of vitamin D produces parathyroid hyperfunction as a result of defective absorption of calcium as suggested by Albright and associates (1 2) or by some more direct means is undecided But it seems likely that a primary disorder of vitamin D metabolism will prove responsible directly or indirectly for most of the renal abnormalities of refractory rickets and osteomalacia.

POTASSIUM DEFICIENCY AND RENAL TUBULAR DISORDERS

The development of a severe potassium deficiency in the experimental animal (153) or in man (70 172 188 204) may be accompanied by a decrease of the glomerular filtration rate and a moderate degree of nitrogen retention These changes are reversed after correction of the potassium deficit and nothing is known of the mechanisms immediately responsible for their production In addition to these apparently haemodynamic changes potassium depletion may cause various functional tubular disorders as well as structural changes in the renal tubular cells (146) The so called nephropathy of potassium depletion (172) is unique among the clinical disorders of renal tubular function in that many of its component functional abnormalities can be reproduced experimentally by inducing potassium depletion in man (37 76) rats (104 200) and dogs (151 198) Moreover considerable insight into the nature of the changes occurring in tubular cells of the potassium depleted kidney can be gained by the *in vitro* study of potassium depleted slices of renal tissue (4 214)

The various renal changes which have been ascribed to potassium deficiency are listed in Table 4 which is meant to be comprehensive As yet, not all the effects listed have been adequately verified and some of the findings in animals (e g renal hypertrophy and the reduced capacity to excrete a water load) may not be directly applicable to man Consequently discussion will be restricted to changes of clinical significance

It has been inferred, from the results of metabolic balance studies in man (24) and in the rat (42), that when an extracellular alkalosis develops with potassium deficiency it is associated with an intracellular acidosis The direct analysis of muscle from potassium depleted rats has revealed a decrease of intracellular pH (93) Recent *in vitro*

studies of the potassium and bicarbonate content of renal slices (4) indicate that the concentration of hydrogen ions within renal cells is increased by potassium depletion and it can be demonstrated that the secretion of hydrogen ion by the renal tubules is increased in potassium deficient states (175). In the light of these various studies it becomes possible to describe comprehensively if tentatively the metabolic state in potassium deficiency and to resolve some apparently anomalous features of renal function in this condition. The extracellular alkalosis is of extrarenal origin (163) and results from a shift of H from the extracellular fluids to cells in partial replacement of lost cellular K (42); the renal tubular cells sharing in the intracellular acidosis (4) increase the secretion of H^+ in exchange for Na^+ reabsorbed from the glomerular filtrate (16); the latter process effects the almost complete reabsorption of the greatly increased filtered load of HCO_3^- and thereby maintains the elevated plasma level of bicarbonate (175). It follows that a metabolic alkalosis of the extracellular fluid is associated with the excretion of what appears to be an inappropriately acid urine—the paradoxic aciduria found clinically with gastric alkalosis (115) and other conditions leading to the alimentary loss of potassium (32). This supposed paradox is to be regarded as a strictly physiologic response of the renal tubule that maintains the newly established ionic equilibria between the intracellular and extracellular fluids.

It has also been shown in man (76) and rat (43) that potassium depletion is associated with a reduced renal capacity to excrete an administered load of sodium bicarbonate; the consumption of alkali in such circumstances may produce severe alkalosis. This observation may apply to patients with pyloric obstruction in whom a potassium deficiency commonly develops (51) and who may increase the alkali intake because of increasing pain. The ensuing severe alkalosis could conceivably be of importance in the production of renal damage; a combination of severe alkalosis with potassium deficiency may account for the mental symptoms sometimes seen with pyloric stenosis and which are not readily explained by the alkalosis alone (219a). Although the consumption of large amounts of alkali and the development of metabolic alkalosis appear to be without deleterious effects on the healthy kidney (219a) it cannot safely be assumed that the same is true of a kidney already damaged by potassium deficiency.

The intracellular acidosis of potassium depletion appears to be directly responsible for two other changes of renal tubular function

(146) Of these the reduced urinary output of citric and other organic acids (43 76 87) seems to be without clinical significance (76) The other concerns the excretion of ammonia and it may be regarded as an adaptation whereby the kidney is enabled to excrete the acid products of metabolism despite a reduced capacity to produce urine of

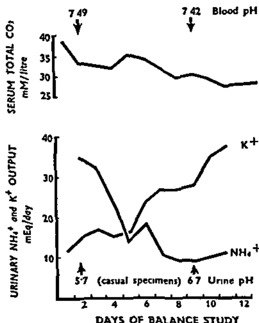


FIG 4—Metabolic alkalosis of potassium depletion and its spontaneous correction by retention of dietary potassium in patient with mitral valvulotomy potassium deficiency due to anorexia and use of mercurial diuretics Fall in blood pH and serum total CO was accompanied by fall in urinary ammonia output urine was collected directly into acid to obtain accurate figures for ammonium output, and output of bicarbonate was therefore not measured (Unpublished observations of Mahler and Stanbury)

maximal acidity In potassium deficiency resulting from alimentary losses (188 228) in experimental potassium deficiency (37) and in primary aldosteronism (69 146) the urinary output of ammonia has been found disproportionately high for the pH of the urine excreted of the H^+ excreted a greater proportion than normal is excreted as NH_4^+ and less as titratable acid Correction of the potassium deficiency is associated with a rapid fall in ammonia output, even though the pH

studies of the potassium and bicarbonate content of renal slices (4) indicate that the concentration of hydrogen ions within renal cells is increased by potassium depletion and it can be demonstrated that the secretion of hydrogen ion by the renal tubules is increased in potassium deficient states (175) In the light of these various studies it becomes possible to describe comprehensively if tentatively the metabolic state in potassium deficiency and to resolve some apparently anomalous features of renal function in this condition The extracellular alkalosis is of extrarenal origin (163) and results from a shift of H from the extracellular fluids to cells in partial replacement of lost cellular K (42) the renal tubular cells sharing in the intracellular acidosis (4) increase the secretion of H^+ in exchange for Na^+ reabsorbed from the glomerular filtrate (16) the latter process effects the almost complete reabsorption of the greatly increased filtered load of HCO_3^- and thereby maintains the elevated plasma level of bicarbonate (175) It follows that a metabolic alkalosis of the extracellular fluid is associated with the excretion of what appears to be an inappropriately acid urine—the paradoxic aciduria found clinically with gastric alkalosis (115) and other conditions leading to the alimentary loss of potassium (32) This supposed paradox is to be regarded as a strictly physiologic response of the renal tubule that maintains the newly established ionic equilibria between the intracellular and extracellular fluids

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lar acidosis since an increase of phosphate clearance is usual with metabolic acidosis Its rapid reversibility with potassium replacement would be compatible with this explanation but its origin in the structural changes of the proximal tubule cannot be excluded

Other renal effects of clinical significance appear to involve functions of both the proximal and distal parts of the nephron (146) Since they may be associated with clinical symptoms and with demonstrable histologic changes they are perhaps better regarded as pathologic rather than as adaptive sequels of the potassium depletion

Although structural changes are frequently found in the proximal tubules evidence of impaired proximal tubular function is scanty Elimination of p aminohippurate (PAH) is depressed whether measured by its renal clearance its extraction ratio or its maximal rate of excretion (T_mPAH) (188) replacement of potassium has been followed by an increase in the clearance of PAH (172) It is likely that the impaired PAH excretion is a direct result of a reduced intracellular potassium concentration and not of structural or haemodynamic renal changes *In vitro* studies by Taggart and co workers (214) indicate that renal slices leached of potassium accumulate little PAH when incubated in a potassium free medium maximal accumulation depends on a normal tissue content of potassium

Polyuria accompanied by thirst may be a conspicuous feature of potassium deficiency in man (41 75 86 139 205) and it occurs regularly in the potassium depleted dog (151 198) and rat (31 104 146 200) It may also be inferred that development of potassium deficiency was involved in the production of the diabetes insipidus like syndrome in dogs treated with desoxycorticosterone acetate (DCA) (83 152) Since the original observations of Schwartz and Relman (188) it has been established that the polyuria of potassium deficiency is due at least in part to an impaired renal capacity to produce osmotically concentrated urine and a hyposthenuria or isosthenuria that depends on the body content of potassium has been observed in a variety of conditions (Table 4) Our most impressive personal experience of this occurrence was in a patient with the genetically determined adult Fanconi syndrome who appeared to have a congenital water losing renal defect or renal diabetes insipidus and in whom osteomalacia and potassium deficiency developed at the age of 35 (Fig 6) The rapidity with which the osmolar concentrating capacity may improve after a positive potassium balance is established may be seen in Figure 6

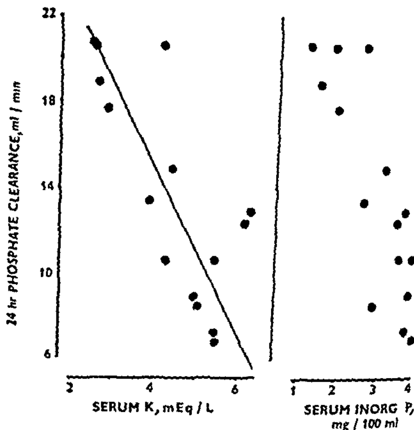


FIG 5—Effects of potassium replacement on renal phosphaturia in patient with chronic pyelonephritis and potassium depletion (modified from ref 139) On administration of potassium bicarbonate plasma potassium level increased with its cumulative positive balance and serum phosphate level increased in roughly parallel fashion creatinine clearance did not change materially Renal clearance of phosphate normally increases with plasma phosphate level (see Fig 2) in this case converse relation obtained indicating that replacement of potassium changed renal tubular handling of phosphate

of the extracellular fluid is falling simultaneously (Fig 4) The facilitation of ammonia excretion can probably be attributed to an increased content of glutaminase and D-amino oxidase in the cells of the renal tubule (109) there are reasons to believe that the adaptive enzymic changes are related to the reduced intracellular pH (49)

Conceivably the renal phosphaturia observed in 1 patient with severe potassium depletion (139) (Fig 5) was also a result of intracellu

since the age of 12 but no other symptoms in early life osteomalacia and potassium deficiency were found at the age of 35 O Osmolarity of whole 24 hour urine specimen numbers in circles days of potassium replacement ● Short urinary collection periods after intramuscular administration of 5 to 10 units of pitressin tannate

When patient was potassium deficient there was no response to pitressin as soon as potassium replacement began the osmolarity of 24 hour specimens increased On day 11 when the potassium deficit was completely made up response to pitressin was demonstrable although urinary osmolarity remained below that of plasma (renal diabetes insipidus) Patient s younger sister with same type of renal lesion but without potassium deficiency responded in manner similar to that shown in right half of figure Full renal and metabolic data in Stanbury (204)

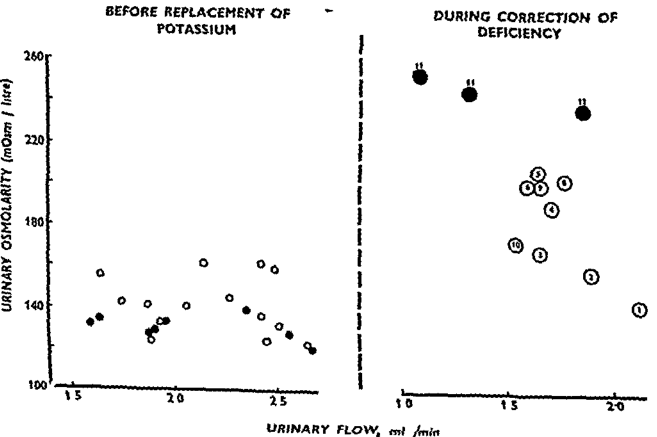


Fig 8.—Water losing renal lesion in adult Fanconi syndrome in 36-year-old female patient with additional impairment of water conservation resulting from potassium deficiency Patient had polydipsia and polyuria at least

Renal Changes	Clinical Disorder Responsible for Deficiency or Experimental Method
Inability to establish minimal urinary pH relative increase in urinary NH_4	Experimental deficiency in man continued ingestion of NH_4Cl and diabetic acidosis (37) steatorrhoea (228) primary aldosteronism (46 69 75 145)
Decreased urinary citric and other organic acids	Experimental deficiency in man (76 87) and in rat (43 76)
Increased tubular reabsorption of HCO_3 and decreased capacity to excrete $NaHCO_3$ load	Experimental deficiency in man (76) and depletion in rat (43 76) various surgical conditions (175) gastric alkalosis (115) ulcerative colitis (32)
Inversion of diurnal excretory rhythm	Chronic pyelonephritis (139) hyperaldosteronism with unilateral renal disease (227)
Impaired capacity to conserve Na with low dietary Na	Renal tubular acidosis (139) Fanconi syndrome in adult (204)
Renal phosphaturia	Renal tubular acidosis (139) (see Fig 5)
Amino-aciduria (tubular[?])	Alimentary losses (64)
Structural changes in renal tubules	
Vacuolar (hydropic) nephropathy of proximal tubule	In various alimentary renal and adrenocortical disorders experimental deficiency in rat (84 85)
Granular degeneration and atrophy of cells in distal and collecting tubules	Alimentary losses (119) experimental deficiency in rat (146 200)
Renal weight increase	Experimental depletion in rat (31 200)
Histochemical changes in renal tubules	
Increased esterase in proximal tubules	Experimental deficiency in rat (137)
Decreased DPN diaphorase and increased TPN diaphorase in medulla and papilla	Experimental deficiency in rat (137)
Biochemical changes in renal tubules	
Decreased capacity to accumulate p-aminohippurate	Kidney slices depleted of K by leaching (214)
Intracellular acidosis	Leached kidney slices reversible by metabolism (4)
Increased glutaminase d-amino oxidase and carbonic anhydrase	Experimental deficiency in rat (109)

Such rapid improvement appears to be usual (139 205) but it may be months before a completely normal concentrating capacity is regained (172 188)

It may be recalled that the diabetes insipidus like syndrome of DCA treated dogs was originally ascribed to a primary polydipsia (83 152) It is the author's clinical impression that a primary thirst, the mechanism of which is obscure can occur as a symptom of potassium deficiency (86 139) and that polydipsia may contribute to the production of the exceedingly high urinary volumes observed in certain cases For instance it is difficult to explain the daily output of 4 to 6 litres in some patients with potassium depletion due to hyperaldosteronism (90 138 227) for most of these patients when tested with vasopressin, can elaborate urine with a specific gravity at least as high as 1 010 The situation may be compared with that found in the syndrome of renal tubular acidosis in which it is not uncommon to find isosthenuria (max sp gr 1 010) in association with normal or only slightly reduced glomerular filtration rates such patients virtually never produce so

TABLE 4 —Renal Effects of Potassium Deficiency

Renal Changes	Clinical Disorder Responsible for Deficiency or Experimental Method
Glomerular changes	
Lowered glomerular filtration rate and urea clearance	Alimentary losses (172 188) nephrotic syndrome treated with cation exchange resins (205) Fanconi syndrome in adult (204) renal disease of uncertain nature (70) primary aldosteronism (138) K depletion cause unknown (116) experimental K depletion in rat (153)
Proteinuria (?)	Present in most severe cases (Glomerular [?] tubular [?])
Impaired capacity to excrete water load	Experimental depletion in rat (31) but not necessarily in man (116 139)
Functional changes in renal tubules	
Hyposthenuria or isosthenuria pitressin resistant	Alimentary losses (172 188) primary aldosteronism (41 69 76) renal tubular *acidosis* (139) nephrotic syndrome treated with cation exchange resins (205) Lignac Fanconi syndrome (*see* Table 1) Fanconi syndrome in adult (*see* Fig 6) experimental depletion in rat (104 146) and probably in dog (151 152 199)
Impaired p aminohippurate extraction and excretion	Alimentary losses (172 188)



PLATE 1 —Vacuolar nephropathy of potassium depletion in male patient with malignant hypertension rendered fatally deficient in potassium by therapy with cation exchange resins (× 320) Appearance is identical with that of case of cystinosis reported by Gatzimos *et al* (94)

great an urinary volume In contrast a patient with hyperaldosteronism studied in this laboratory by Wrong (227) excreted daily up to 4 5 litres of urine with an osmolarity between 180 and 210 mOsm per litre after deprivation of water or administration of vasopressin he was able to produce urine with an osmolarity of 310 mOsm per litre at a rate of about 1 2 ml per minute (equivalent to less than 2 litres daily) Were this degree of limitation of osmolar concentration alone responsible for the polyuria one would not expect the urinary volume to exceed 2 to 3 litres daily This is a fascinating aspect of potassium deficiency to which more attention should be directed It must be borne in mind that some symptoms of primary aldosteronism which we tend to ascribe to potassium deficiency may prove to be due to the abnormal production of aldosterone itself

The pertinence of the last remark is evident on considering the mechanisms of defective urinary acidification in simple potassium deficiency and in primary aldosteronism In a study of experimental potassium depletion Clarke and co workers (37) observed the response of the urinary pH to the administration of ammonium chloride subjects who were previously able to produce a minimal pH of 4 5 could not achieve values much lower than pH 5 4 when their potassium deficiency was 300 mEq This constitutes a considerable reduction of the tubular (presumably distal) capacity to establish a gradient of hydrogen ion between the plasma and the urine and it accounts for the low urinary output of titratable acid in potassium deficiency Defective acidification of this degree has been observed in potassium deficiency associated with various experimental and clinical states (Table 4) Several patients with hyperaldosteronism have shown a minimal urinary pH close to 6 5 after stimulation with ammonium chloride (46 69 75 145 227) it has been natural to ascribe the renal defect to the associated potassium depletion of this disease It seems however that this assumption is unwarranted In some patients it has proved possible to replenish body potassium without restoring a normal capacity for urinary acidification (69 145) after removal of the aldosterone secreting tissue this function was regained without change in the external potassium balance (69 145 227) It would thus appear possible that the more severe impairment of acidification in primary aldosteronism is due to aldosterone itself rather than to potassium deficiency It may be assumed that aldosterone influences the cation exchange mechanism whereby sodium ion is reabsorbed in exchange for K^+ or H^+ secreted by

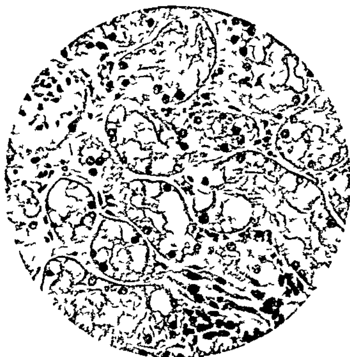


PLATE 1—Vacuolar nephropathy of potassium depletion in male patient with malignant hypertension rendered fatally deficient in potassium by therapy with cation exchange resins (× 320) Appearance is identical with that of case of cystinosis reported by Gatzimos *et al* (94)

the tubules (16) by facilitating the tubular secretion of potassium ion, it may inhibit the secretion of hydrogen ion and so prevent urinary acidification. This action of aldosterone may also account for the fact that patients with primary aldosteronism continue to excrete potassium despite established potassium depletion. In simple potassium deficiency urinary potassium excretion is minimal, and stimuli which normally promote its excretion become relatively ineffective (37 76).

The three renal tubular functions—excretion of PAH, establishment of a hydrogen ion gradient, and osmotic concentration of the urine—which are pathologically defective in potassium deficiency all necessitate the expenditure of energy by the tubular cells. We would agree with the suggestion (146) that impaired formation of phosphate-bond energy by the potassium-deficient cells (see references in 139 169) may account for the tubular defects. The same factor could account for the impaired conservation of sodium chloride observed in 2 patients (139 206) and it is to be expected that other tubular defects will be found if assiduously sought. What is the significance of the structural changes developing in the renal tubules with potassium depletion? These will not be discussed in detail as many descriptions have been published of the lesions occurring in animals (84 85 146 187 199 200) and man (119 131 164 172). They include granular hydropic vacuolar and desquamative lesions of the convoluted tubules and granular degeneration, atrophy and dilatation of the distal and collecting tubules. An example of the typical changes is shown in Plate 1. Some patients with potassium deficiency show no structural lesions in the tubules. It seems unlikely that the structural changes are the *cause* of the accompanying functional defects. There is evidence that the continued provision of phosphate bond energy is necessary to preserve the structural integrity of cells; the functional and structural changes of potassium deficiency are probably parallel, rather than consecutive and dependent, effects.

It remains to be determined whether a severe and prolonged potassium deficiency can result in permanent renal changes. It seems likely that it can (205) but conclusive evidence is lacking. Several investigators have suggested that a potassium deficiency may predispose to the development of pyelonephritis (69 88 146). This suggestion, based partly on findings in a small number of patients with primary aldosteronism, should be treated with caution. Pyelonephritis is the commonest of the chronic renal diseases; there is some evidence to suggest that

certain patterns of renal disease can produce an apparently irreversible secondary hyperaldosteronism (206 227) Primary renal disease is a prominent feature among the causes of potassium depletion (Table 4) and the latter may then be responsible for the production of additional renal changes (139 205) The interrelations between renal disease potassium deficiency, and primary and secondary adrenocortical hyperfunction are intricate and manifold some aspects of the problem are discussed elsewhere (209)

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